의학박사 학위논문

Significance of the *BRAF* and *RAS* mRNA expression level in papillary thyroid carcinoma: An analysis of The Cancer Genome Atlas data

갑상선유두암에서의 *BRAF* 와 *RAS* 유전자 mRNA 발현양의 임상적 의의: The Cancer Genome Atlas 분석

2017년 6월

서울대학교 대학원

의학과 외과학 전공

채 영 준

갑상선유두암에서의 *BRAF* 와 *RAS* 유전자 mRNA 발현양의 임상적 의의: The Cancer Genome Atlas 분석

지도교수 이 규 언

이 논문을 의학박사학위논문으로 제출함

2017년 6월

서울대학교 대학원

의학과 외과학 전공

채 영 준

채영준의 의학박사학위논문을 인준함

2017년 6월

위 원 장 한 원 식 (인)

부위원장 이 규 언 (인)

위 원 박 영 주 (인)

위 원 천 기 정 (인)

위 원 박 원 서 (인)

Significance of the *BRAF* and *RAS* mRNA expression level in papillary thyroid carcinoma: An analysis of The Cancer Genome Atlas data

by Young Jun Chai, M.D.

(Directed by Kyu Eun Lee, M.D., Ph.D.)

A Thesis Submitted to the Department of Surgery in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Medicine (Surgery) at Seoul National University College of Medicine, Seoul, Korea

June, 2017

Approved by thesis committee

Professor _____ Chairman

Professor _____ Vice Chairman

Professor _____

Professor _____

Professor _____

Abstract

Significance of the *BRAF* and *RAS* mRNA expression level in papillary thyroid carcinoma: An analysis of The Cancer Genome Atlas data

Young Jun Chai

Medicine (Surgery)

The Graduate School

Seoul National University

Background and Aim

BRAF and *RAS* mutations are the two most common mutations in papillary thyroid carcinoma (PTC), and *BRAF* mutation is associated with high-risk prognostic factors. However, the significance of the mRNA expression level of the two genes in PTC remains unknown. In this study, the significance of *BRAF* and *RAS* mRNA

expression levels were investigated by analyzing PTC data from The Cancer Genome Atlas (TCGA) database.

Methods

Data from 499 patients were downloaded from the TCGA database. After excluding other PTC variants, total 353 cases of classic PTC, including 193 cases with *BRAF*^{V600E} and 160 cases with the wild-type *BRAF* were selected. mRNA abundances were measured using RNA-Seq with the Expectation Maximization algorithm.

Results

None of the clinicopathological factors, including age, gender, thyroiditis, extrathyroidal extension, tumor size, AJCC stage, recurrence, or vital status, differed significantly between the *BRAF*^{V600E} and wild-type *BRAF* patients. The mean *BRAF* mRNA level was significantly higher in *BRAF*^{V600E} patients than in patients with wild-type *BRAF* (197.6 vs. 179.3, p = 0.031). In wild-type *BRAF* patients, the mean *BRAF* mRNA level was higher in cases with a tumor > 2 cm than those with a tumor ≤ 2.0 cm (189.4 vs. 163.8, p = 0.046), and was also higher in cases with lymph node metastasis than in those without lymph node metastasis (188.5 vs. 157.9, p =0.040). Within *BRAF*^{V600E} patients, higher *BRAF*

mRNA expression was associated with extrathyroidal extension (186.4 vs. 216.4, p = 0.001) and higher T stage (188.1 vs. 210.2, p =0.016). In $BRAF^{V600E}$ patients, higher *NRAS* mRNA expression count was associated with presence of thyroiditis (905.0 vs. 785.6, p = 0.001), extrathyroidal extension (921.6 vs. 826.5, p = 0.007), higher T stage (905.2 vs. 829.3, p = 0.024), and lymph node metastasis (906.0 vs. 822.0, p = 0.017). The mRNA expression count of *KRAS* was higher in the patients with extrathyroidal extension (738.8 vs. 647.9, p = 0.001), higher T stage (724.2 vs. 649.8, p = 0.005), lymph node metastasis (717.5 vs. 653.3, p = 0.018), and higher AJCC stage (721.9 vs. 661.9, p = 0.042).

Conclusions

A higher *BRAF* mRNA expression level was associated with tumor aggressiveness in classic PTC regardless of *BRAF* mutational status. *NRAS* and *KRAS* mutations were associated with tumor aggressiveness in $BRAF^{V600E}$ patients. Evaluation of *BRAF* and *RAS* mRNA level may be helpful in prognostic risk stratification of PTC.

Keyword: thyroid carcinoma, papillary thyroid carcinoma, *BRAF*, *RAS*, *BRAF* mutation, *RAS* mutation

Student Number: 2015-30565

Contents

List of Tables

List of Figures

Introduction

Thyroid carcinoma is the most common endocrine malignancy, and its incidence has increased rapidly over the past few decades. In 2014, an estimated 62,980 new patients were expected in the United States [1], and the incidence has increased 3-fold over the past 30 years. In general, thyroid carcinoma can be classified into follicular epithelial cell-derived papillary thyroid cancer (PTC), follicular thyroid cancer (FTC), anaplastic thyroid cancer, and para-follicular C-cell derived medullary thyroid cancer (MTC), which account for approximately 80, 15, 2, and 3% of all thyroid malignancies, respectively [2]. The prognosis is generally excellent, and the mortality is as low as 0.5 cases per 100,000 people [3]. However, a subset of thyroid carcinomas has a poor prognosis that is not adequately explained by traditional staging systems. Recent advances in cancer genetics provide new opportunities for improved assessment, and the molecular markers now available represent an effective strategy for the diagnosis and prognostication of thyroid carcinoma.

BRAF somatic mutations, the most extensively investigated molecular markers, are the most common genetic alterations in PTC. *BRAF* mutation occurs exclusively in about 45% of PTC; it does not occur in FTC, MTC, and benign thyroid tumors [4]. One somatic mutation, $BRAF^{V600E}$, results in the substitution of a valine by a glutamate at reside 600 and is associated with adverse prognostic factors such as extrathyroidal extension (ETE), lymph node metastasis, advanced American Joint Committee on Cancer (AJCC) stage, recurrence, distant metastasis, and poor survival [5-8]. This is explained

by the fact that *BRAF*^{V600E} mutation, via activation of the MAP kinase pathway, causes loss of expression of thyroid genes and refractoriness to radioiodine, as well as up-regulation of angiogenic and tumor-promoting molecules [9, 10]. On the other hand, the incidence of the *BRAF*^{V600E} mutation in classic PTC varies widely between studies (38-83%) [4], and its positive predictive value for cancer recurrence is only 28% [11]. Furthermore, the results of several studies have challenged the notion that the *BRAF*^{V600E} mutation is a valuable prognostic marker [12, 13]. Consequently, the prognostic use of the *BRAF*^{V600E} mutation may not be generalizable to all clinical settings.

RAS mutations are the second most common genetic alterations in thyroid carcinoma, and they are related to the tumorigenesis of FTC and follicular variant PTC. There are three isoforms encoded by the *RAS* gene; *NRAS*, *HRAS*, and *KRAS*. *RAS* mutations play a key role in the transduction of signals from tyrosine kinase and G protein-coupled receptors to effectors of the MAPK and PI3K-AKT signaling pathways, which mediate cell differentiation, proliferation, and survival [14]. Under normal conditions, *RAS* activity is tightly regulated by GTP-mediated hydrolysis of activated GTP-bound *RAS* to inactivated GDP-bound *RAS* [15]. Point mutations produce oncogenic alleles of *RAS* that exhibit either increased affinity for GTP or inhibition of autocatalytic GTP-ase function. Both mechanisms result in constitutive, aberrant activation of the downstream MAPK and PI3/AKT signaling pathways, a critical event in thyroid tumorigenesis [16, 17]. *RAS* mutations are found in 10 to 56% of FTC [18, 19], and rarely found in PTC. The incidence of *RAS* mutations in FTC is more common in Asia than in Western countries, whereas the incidence of *RAS* mutations in PTC is more

common in Western countries than in Asia [20]. The *RAS* mutations are associated with better differentiation of thyroid cancer and the expression of iodide-handling genes in *RAS* mutated PTC are normal or near-normal [21, 22].

Several studies have suggested that the behavior of PTC is influenced not only by *BRAF* mutation status, but also by the expression level of the Braf mutant protein or the percentage of *BRAF* mutant alleles. One study reported that higher expression of Braf mutant protein predicts aggressive tumor behavior in PTC [23]. Other studies assessed the correlation between the percentage of *BRAF* mutant alleles and clinical outcomes in PTC [24, 25]. The results showed that higher percentages of *BRAF* mutant alleles were associated with poor prognostic factors such as older age, larger tumor size, ETE, and higher recurrence rate.

The Cancer Genome Atlas (TCGA) project, a collaboration between the National Cancer Institute and National Human Genome Research Institute, has started in 2006 and generated comprehensive, multi-dimensional maps of the key genomic changes in 33 types of cancer. The TCGA dataset, 2.5 petabytes of data describing tumor tissue and matched normal tissues from more than 11,000 patients, is publically available and has been used widely by the research community. Multiple studies have been performed using this powerful open resource, and several studies of thyroid carcinoma using this data resource have been reported recently [24, 26, 27]. The TCGA portal provides an enormous amount of information, including data regarding microsatellite instability, DNA sequencing, miRNA sequencing, protein expression, mRNA sequencing, DNA methylation, copy number variation, clinical information, and clinical images.

In this study, the mRNA expression level of *BRAF* and *RAS* was analyzed in cases of

PTC from TCGA database. And the significance of the *BRAF* and *RAS* mRNA expression level in $BRAF^{V600E}$ and wild-type *BRAF* PTC was investigated to evaluate the prognostic value.

Materials and Methods

Data acquisition

As of March 2015, TCGA group made available multiple types of genomic data regarding thyroid carcinoma, including somatic mutation, exome sequencing, methylation array, mRNA expression count, microRNA expression count and clinical information. The data on somatic mutation, mRNA expression count, and clinical information were downloaded from the TCGA data portal (https://tcga-data.nci.nih.gov/tcga/tcgaDownload.jsp). All patient information was anonymized and de-identified in this database. According to TCGA publication guidelines (http://cancergenome.nih.gov/publications/publicationguidelines), there are no restrictions on the publication of these somatic mutation and mRNA sequencing data and no specific permission is required for investigators to publish papers containing or referring to these data. Somatic mutation data were provided as a mutation call file by the Broad Institute and the Baylor College of Medicine. The Illumina Genome Analyzer was used as the platform for DNA sequencing (Illumina Inc., San Diego, CA, USA). mRNA sequencing data, obtained by Illumina HiSeq 2000 RNA Sequencing Version 2 analysis, were provided by the University of North Carolina. mRNA expression counts were obtained via the TCGA portal and are expressed as RNA-Seq by Expectation Maximization (RSEM) values. RSEM is an accurate software tool for quantifying transcript abundances from RNA-Seq data [28].

After excluding the patients with missing information, the data from a total of 499 patients were downloaded. The examples of the molecular test and pathologic result were shown in Figure 1. Every scanned original pathologic report file was reviewed and the information was revised when there was missing information. In cases of multifocal PTC, the largest tumor was analyzed.

Figure 1. Example of molecular test (A) and pathologic report (B) uploaded in the TCGA database

(A)

PATIENT HISTORY:
CHIEF COMPLAINT/ PRE-OP/ POST-OP DIAGNOSIS: Metastatic thyroid cancer.
PROCEDURE: Total thyroidectomy, excision lymph node.
SPECIFIC CLINICAL QUESTION: Not answered.
OUTSIDE TISSUE DIAGNOSIS: Not answered.
PRIOR MALIGNANCY: Not answered.
CHEMORADIATION THERAPY: Not answered.
ORGAN TRANSPLANT: Not answered.
IMMUNOSUPPRESSION: Not answered.
OTHER DISEASES: Not answered.

ICD-O-3
carcinoma, follicular, NOS 8330/3
Site: thyroid, NOS C73.9
hw 11/22/11

ADDENDA:
Addendum
Molecular Anatomic Pathology Testing:

Block 1D:

A. ***HRAS* codon 61 mutation IDENTIFIED.**
B. **Mutations in *BRAF, NRAS61, and KRAS12/13* NOT identified.**

Note:
DNA was extracted in the amount sufficient for testing.

Mutations in either *BRAF* or *RAS* genes or *RET/PTC* rearrangements are found in more than 70% of papillary thyroid carcinomas (1). *BRAF* V600E mutation has been associated with more aggressive behavior of papillary carcinoma (2, 3). The association between *BRAF* V600E mutation and features of tumor aggressiveness have also been observed in papillary microcarcinomas (4). Mutations in the *RAS* genes or *PAX8/PPARg* rearrangement occur in ~70% of follicular thyroid carcinomas and with lower frequency in oncocytic (Hürthle cell) carcinomas (5). Regarding the specificity of these mutations for cancer, *BRAF* V600E mutation and *RET/PTC* and *PAX8/PPARg* rearrangements are overall specific for malignancy in the thyroid, although they have been reported with a very low frequency in benign thyroid lesions (6). *RAS* mutations occur in malignant and benign thyroid tumors, being found in ~40-50% of follicular and anaplastic carcinomas, 30-40% of follicular adenomas and 10-15% of papillary carcinomas (6).

1. Adeniran AJ, et al. Correlation between genetic alterations and microscopic features, clinical manifestations, and prognostic characteristics of thyroid papillary carcinomas. *Am J Surg Pathol* 2006, 30:216-222
2. Xing M, et al. *BRAF* mutation predicts a poorer clinical prognosis for papillary thyroid cancer. *J Clin Endocrinol Metab* 2005, 90:6373-6379.
3. Elisei R, et al. *BRAF* V600E mutation and outcome of patients with papillary thyroid carcinoma: a 15-year median follow-up study. *J Clin Endocrinol Metab* 2008, 93:3943-3949.
4. Lee X, Gao M, Ji Y, Yu Y, Feng Y, Li Y, Zhang Y, Cheng W, Zhao W. Analysis of differential BRAF(V600E) mutational status in high aggressive papillary thyroid microcarcinoma. *Ann Surg Oncol.* 2009 Feb;16(2):240-5.
5. Nikiforova MN, et al. *RAS* point mutations and*PAX8-PPARγ* rearrangement in thyroid tumors: Evidence for distinct molecular pathways in thyroid follicular carcinoma. *J Clin Endocrinol Metab* 2003, 88: 2318-26.
6. Nikiforov YE. Recent developments in the molecular biology of the thyroid. In: Lloyd RV., ed. *Endocrine Pathology: Differential Diagnosis and Molecular Advances*. Humana Press, Totowa, 2004, 191-209.

Sample Preparation and Procedure
For paraffin-embedded surgical specimens, manual microdissection was performed to collect tumor tissue. Specimens with the minimum of 50% of tumor cells in a microdissection target are accepted for the analysis. Optical density readings were obtained. Real-time PCR was performed on the , atform to amplify BRAF, NRAS codon 61, HRAS codon 61, and KRAS codons 12/13 sequences. Post-PCR melting curve analysis was used to detect possible mutations. If required, the mutation type was confirmed by Sanger sequencing of the PCR product on DNA from samples positive for each of these mutations was used as positive controls. Amplification at 35 cycles or earlier was considered sufficient for the analysis. The limit of detection is approximately 10% of alleles with mutation present in the background of normal DNA and RNA.

Notified
Procured and passed before final path report review.
hw 11/22/11

Criteria	Yes	No
Diagnosis Discrepancy	X	
Primary Tumor Site Discrepancy		X
ISPAA Discrepancy		X
Prior Malignancy History		X
Case is Ineligible		X
Reviewer Initials	DISQUALIFIED	

Notifiable

(B)

Addendum
Molecular Anatomic Pathology Testing:

Block D:

A. Loss of heterozygosity is IDENTIFIED at chromosome 22q.
B. Fractional Allelic Loss is 9% (1 locus with loss / 11 informative loci).
C. LOH at the VHL gene locus (3p.26) is NOT identified.

Note:
Loss of tumor suppressor gene alleles is known to occur in thyroid follicular neoplasias and to correlate with tumor progression. As reported in the literature, widely invasive carcinomas and anaplastic carcinomas frequently have a mean fractional allele loss of ~50% (1). Adenomas typically have lower frequency of LOH, frequently fewer than 10%. Minimally invasive and angioinvasive follicular carcinomas typically have intermediate results (1,2). Some studies have shown that LOH at the VHL gene locus is specific for malignancy and is associated with poor prognosis (2,3). However, not all tumors reveal such a correlation and, therefore, the LOH profile should be interpreted in the context of the cytologic and histologic findings and the patient's clinical history.

Sample Preparation and LOH Analysis
For cytology samples, extraction of DNA was performed from the fluid or sample provided. For surgical specimens, manual microdissection was performed to include neoplastic tissue and normal adjacent tissue. Specimens with the minimum of 50% of tumor cells in a microdissection target are accepted for the analysis. DNA was isolated using standard laboratory procedure. Optical density readings were obtained.
Fourteen microsatellite markers (listed below) that have been previously found to be involved in thyroid neoplasia and co-localize with known tumor suppressor genes were used for analysis. PCR was performed using fluorescently labeled primers and the products of amplification were detected using capillary electrophoresis on ABI3730 platform. Relative fluorescence was determined for individual alleles and the ratio of peaks was calculated (GeneMappper ABI 3730). Normal tissue was examined to determine whether the patient is heterozygous at the marker (genetically informative) and neoplastic tissue was then analyzed to detect loss of heterozygosity. Thresholds for significant allelic imbalance were determined using normal (non-neoplastic) specimens for every marker. Loss of heterozygosity was determined using $(N^{L} / N^{S})/ (T^{L}/T^{S})$ *formula and reported when allelic ratio for a particular marker was below 0.5 or above 2.0. When normal tissue was not available, peak height ratios falling outside of 2 SDs beyond the mean for each polymorphic allele paring were assessed as showing loss of heterozygosity.*

D1S1161	*1p*	*1p35.1*
D1S407	*1p*	*1p36.21*
D3S1038	*VHL*	*3P25.3*
D3S1539	*VHL*	*3p26.3*
D5S659	*APC*	*5q23.2*
D5S1384	*APC*	*5q23.3*
D9S251	*CDKN2/p16*	*9p21.3*
D9S1748	*CDKN2/p16*	*9p22.2*
D10S1171	*PTEN*	*10q23.31*
D10S1173	*PTEN*	*10q23.31*
D17S1844	*p53*	*17p13.1*
D17S786	*p53*	*17p13.1*
D22S1150	*NF2*	*22q12.2*
D22S268	*NF2*	*22q12.2*

References

1. Hunt JL, et. al. Molecular evidence of anaplastic transformation in coexisting well-differentiated and anaplastic carcinomas of the thyroid. American Journal of Surgical Pathology 2003;27:1559-64.
2. Hunt JL, et. al. A novel microdissection and genotyping of follicular-derived thyroid tumors to predict aggressiveness. Human Pathology 2003;34:375-80.
3. Hunt JL, et. al. Loss of heterozygosity of the VHL gene identifies malignancy and predicts death in follicular thyroid tumors. Surgery. 2003 Dec:134(6):1043-7; discussion 1047-8.2003;134:1043-7.

FINAL DIAGNOSIS:

THYROID GLAND, TOTAL THYROIDECTOMY (42.5 GRAMS) –
A. ANGIOINVASIVE FOLLICULAR CARCINOMA (2.8 CM), IN THE LEFT LOBE.
B. MULTIFOCAL CAPSULAR INVASION IS IDENTIFIED.
C. TUMOR IS <0.1 CM FROM THE MARGIN.
D. BACKGROUND THYROID WITH NODULAR HYPERPLASIA.
E. pT2 NX M1 (right femur.

CASE SYNOPSIS:

SYNOPTIC DATA - PRIMARY THYROID TUMORS

SPECIMEN TYPE:	Total Thyroidectomy
TUMOR SITE:	Left Lobe
TUMOR FOCALITY:	Unifocal
TUMOR SIZE (largest nodule):	Greatest Dimension: 2.8 cm
HISTOLOGIC TYPE:	Other: Follicular carcinoma
PATHOLOGIC STAGING (pTNM):	pT2
	pNX
	Number of regional lymph nodes examined: 0
	Number of regional lymph nodes involved: 0
	pM1
	Site(s) of metastasis: Right femur
MARGINS:	Margin(s) involved by carcinoma
VENOUS/LYMPHATIC (LARGE/SMALL VESSEL) INVASION (V/L):	Present
ADDITIONAL PATHOLOGIC FINDINGS:	Other: Nodular thyroid hyperplasia

Patient selection

Of the 499 cases of PTC, variants type of PTC were excluded, leaving a total of 353 classic PTCs. Subtype classification of the PTC patients was based on a previously published paper by Cancer Genome Atlas Research Network [27]. Follicular variant or tall cell variant PTC was diagnosed when more than 99% of the tumor exhibited a follicular pattern or more than 50% of tall cell features, respectively [27]. Two patients with *BRAF* mutations other than V600E (N581T, V459V) were also excluded. Ultimately, 193 classic PTCs with $BRAF^{V600E}$ and 160 classic PTCs with wild-type *BRAF* were selected for the analysis.

Statistics

Data were analyzed using the R software version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Chi-square tests and Fisher's exact test were used to compare categorical variables. Unpaired two sample t-test and linear regression analysis were performed to compare mRNA expression counts. Recursive Partitioning and Regression Trees were used to calculate the cut-off value of

BRAF^{V600E} mRNA expression above which the expression levels correlate with poor prognosis parameters. The Kaplan-Meier estimator was used for survival analysis. A p-value < 0.05 was considered significant.

Results

The patient data are shown in Table 1. The median follow-up duration was 20.7 months (range, 0.03- 171.70 months). Kaplan- Meier overall survival for the study population based on clinicopathological characteristics were shown in Figure 2. Age ≥ 45 (p < 0.001), higher Tumor stage (T stage, p < 0.001), and higher AJCC stage (p < 0.001) were associated with poorer overall survival. Other clinicopathological characteristics such as gender (p = 0.293), size > 2.0 cm (p = 0.186), ETE (p = 0.14), presence of thyroiditis (p = 0.129), and lymph node metastasis (p = 0.225) were not significantly associated with overall survival.

Table 1. Clinicopathological data of 355 patients

No	ID	Age	Sex	RT	operation	location	ETE	Size	No. meta LN	No. LN	T stage	N stage	M stage	AJCC stage	$BRAF^{V600E}$	Expression	Vital	FU	Recurrence
1	TCGA-4C-A93U	74	F	no	Lobectomy	Rt	yes	6.5	1	41	T4a	N1b	M0	IV	no	185.0242	alive	129	no
2	TCGA-BJ-A0YZ	65	M	no	Lobectomy	Rt+Isthmus	no	3	0	0	T2	Nx	M0	II	no	276.1349	alive	754	no
3	TCGA-BJ-A0Z0	55	M	n.a	total	Rt	no	3.5	0	5	T2	N0	MX	II	no	187.938	alive	419	no
4	TCGA-BJ-A0Z3	33	F	n.a	total	Lt	no	1.3	0	3	T1b	N0	M0	I	yes	176.7898	alive	497	no
5	TCGA-BJ-A0Z5	58	M	n.a	total	Lt	yes	6	4	5	T3	N1a	M0	III	no	202.6724	alive	448	no
6	TCGA-BJ-A0ZA	67	F	no	total	Bilat	no	3	0	2	T2	N0	M0	II	no	183.7191	alive	574	no
7	TCGA-BJ-A0ZB	66	M	no	total	Rt	yes	2.4	6	17	T3	N1b	M0	IV	yes	245.8713	alive	122	no
8	TCGA-BJ-A0ZC	55	M	no	total	Lt	no	0.4	0	0	T1a	Nx	M0	I	no	178.9934	alive	352	no
9	TCGA-BJ-A0ZE	63	F	no	Lobectomy	Rt	no	5.7	0	0	T3	Nx	M0	III	no	122.1105	alive	509	no
10	TCGA-BJ-A0ZJ	36	M	no	total	Bilat	no	2.5	0	8	T2	N1a	M0	I	no	112.1019	alive	538	no
11	TCGA-BJ-A18Y	29	M	no	total	Bilat	no	1.4	0	5	T1b	N0	MX	I	no	140.8557	alive	710	no
12	TCGA-BJ-A18Z	58	M	n.a	total	Bilat	yes	3.6	3	37	T3	N1b	MX	IV	yes	186.959	alive	630	no
13	TCGA-BJ-A190	55	M	no	total	Bilat	no	3.5	0	1	T2	N0	MX	II	no	300.2173	alive	626	no
14	TCGA-BJ-A192	54	F	no	total	Lt	no	5	0	0	T3	Nx	M0	III	no	220.7506	alive	1349	no
15	TCGA-BJ-A28R	38	F	n.a	total	Rt	no	1.5	0	20	T1b	N0	M0	I	yes	237.9503	alive	1282	no
16	TCGA-BJ-A28T	34	F	n.a	total	Bilat	no	1.3	3	9	T1b	N1a	M0	I	no	171.7036	alive	1081	no
17	TCGA-BJ-A28V	79	F	n.a	total	Bilat	no	4.5	0	0	T3	Nx	MX	III	no	142.5628	alive	155	no
18	TCGA-BJ-A28W	32	F	n.a	total	Bilat	no	3	0	4	T2	N0	M0	I	no	64.5161	alive	344	no
19	TCGA-BJ-A28Z	46	F	n.a	total	Lt	yes	1	1	16	T3	N1a	M0	III	no	131.1787	alive	37	no

20	TCGA-BJ-A291	56	F	n.a	total	Rt	no	1.5		0	9	T1b	N0	M0	I	no	79.1396	alive	696	no
21	TCGA-BJ-A2N7	30	F	no	total	Bilat	no	5		0	0	T3	Nx	M0	I	no	174.0475	alive	559	no
22	TCGA-BJ-A2N8	30	F	no	total	Rt+Isthmus	no	1.4		0	2	T1b	N0	M0	I	no	145.7765	alive	504	no
23	TCGA-BJ-A2N9	42	F	no	total	Rt	no	2.5		0	11	T2	N0	M0	I	no	128.9575	alive	623	yes
24	TCGA-BJ-A2n.a	77	M	no	total	Bilat	yes	1.5		0	1	T3	N0	M0	III	yes	201.8809	alive	577	yes
25	TCGA-BJ-A2P4	29	F	n.a	total	Lt	no	3.3		0	0	T2	Nx	M0	I	no	90.6433	alive	214	no
26	TCGA-BJ-A3EZ	51	M	no	total	Rt+Isthmus	yes	2.4		6	42	T3	N1b	M0	IV	yes	238.7848	alive	487	no
27	TCGA-BJ-A3F0	64	F	no	total	Rt	no	1.4		0	0	T1b	Nx	M0	I	no	138.8808	alive	424	no
28	TCGA-BJ-A3PR	69	F	no	total	Rt	yes	2.5		0	6	T3	N0	M0	III	yes	131.0661	alive	471	no
29	TCGA-BJ-A3PT	51	F	no	total	Bilat	yes	1.5		0	0	T3	Nx	M0	III	no	91.4206	alive	709	no
30	TCGA-BJ-A3PU	52	M	no	total	Lt	yes	2.6		7	7	T3	N1a	M0	III	yes	156.9193	alive	567	yes
31	TCGA-BJ-A45C	78	M	no	total	Lt	yes	1		0	2	T3	N0	M0	III	no	142.9565	alive	175	no
32	TCGA-BJ-A45E	46	F	n.a	total	Lt	no	0.5		0	4	T1a	N0	M0	I	no	155.061	alive	639	no
33	TCGA-BJ-A45F	59	F	n.a	total	Rt	no	1.5		0	0	T1b	Nx	M0	I	no	133.6037	alive	105	no
34	TCGA-BJ-A45H	45	M	n.a	total	Bilat	yes	1.5		0	0	T3	Nx	M0	III	no	126.2226	alive	238	no
35	TCGA-BJ-A4O8	47	M	n.a	total	Lt+Isthums	yes	1.5		6	14	T3	N1a	M0	III	yes	140.6567	alive	150	no
36	TCGA-CE-A13K	30	F	n.a	Unreported	Lt	yes	4.5	n.a	n.a		T3	N1	M0	I	no	106.1425	alive	385	no
37	TCGA-CE-A27D	28	F	n.a	total	Unreported	no	3.5		8	8	T2	N1a	M0	I	no	125.4093	alive	374	no
38	TCGA-CE-A3MD	31	M	n.a	Unreported	Rt	no	1.5	n.a	n.a		T2	N0	M0	I	no	96.7453	alive	389	no
39	TCGA-CE-A3ME	51	F	n.a	Unreported	Rt	no	4	n.a	n.a		T2	N0	M0	II	yes	240.249	alive	379	no
40	TCGA-CE-A481	41	F	no	Unreported	Lt	no	1.5	n.a	n.a		T1b	N0	M0	I	no	114.2169	alive	346	no
41	TCGA-CE-A482	27	F	no	Unreported	Rt	no	2	n.a	n.a		T1b	N1	M0	I	no	123.6433	alive	325	no
42	TCGA-CE-A483	34	F	no	Unreported	Lt	no	4		4	7	T2	N1	M0	I	no	229.9494	alive	3	no

43	TCGA-CE-A484	37	F	no	Lobectomy	Rt	no	5		2	2	T2	N1	M0	I	no	362.0167	alive	12	no
44	TCGA-CE-A485	32	M	no	total	Bilat	no	4.5	n.a		n.a	T2	N1	M0	I	no	140.0203	alive	3	no
45	TCGA-DE-A0XZ	65	F	no	total	Rt	no	2		5	7	T1b	N1a	M0	III	yes	139.7975	alive	615	yes
46	TCGA-DE-A0Y2	30	F	no	total	Lt	no	2.3		6	11	T2	N1a	M0	I	no	871.0571	alive	469	no
47	TCGA-DE-A0Y3	60	F	no	total	Bilat	yes	2.5		4	22	T4a	N1b	M1	IV	yes	266.438	alive	1109	no
48	TCGA-DE-A3KN	49	F	no	total	Lt	yes	4.5		5	9	T3	N1b	M0	IV	no	134.3576	alive	482	no
49	TCGA-DE-A4M8	61	F	no	total	Bilat	no	3.2		1	3	T2	N1a	M0	III	no	118.9296	alive	564	no
50	TCGA-DE-A4M9	28	M	no	Lobectomy	Rt+Isthmus	no	3		0	0	T2	Nx	M0	I	no	282.7427	alive	573	no
51	TCGA-DE-A4MA	52	F	no	total	Lt	yes	4.2		7	8	T3	N1a	M0	III	no	120.418	alive	916	no
52	TCGA-DE-A4MB	79	F	no	total	Bilat	yes	4.4		3	36	T3	N1b	M0	IV	no	201.1913	alive	352	no
53	TCGA-DE-A4MC	43	F	no	total	Rt	yes	2.8		1	1	T3	N1b	M0	I	no	264.8154	alive	913	yes
54	TCGA-DE-A4MD	71	M	no	total	Bilat	no	6		9	41	T3	N1b	M0	IV	no	318.8515	alive	764	yes
55	TCGA-DE-A69J	34	F	no	total	Rt	no	2.5		0	0	T2	Nx	M0	I	no	235.7714	alive	447	no
56	TCGA-DE-A69K	58	F	no	total	Rt	yes	3.1		0	4	T3	N0	M0	III	no	131.0558	alive	434	yes
57	TCGA-DE-A7U5	36	F	no	total	Rt	no	2.4		0	8	T2	N0	M0	I	no	190.8726	alive	84	no
58	TCGA-DJ-A13O	56	M	no	total	Rt	no	1.1		0	2	T1b	N0	M0	I	yes	112.5146	alive	428	no
59	TCGA-DJ-A13P	52	F	yes	total	Bilat	yes	1.5		0	0	T3	Nx	M0	III	yes	184.3419	alive	5150	no
60	TCGA-DJ-A13T	37	F	no	total	Bilat	no	2.2		0	7	T2	N0	M0	I	yes	247.3341	alive	1709	no
61	TCGA-DJ-A13U	60	M	no	Lobectomy	Rt+Isthmus	yes	1.1		0	0	T3	Nx	M0	III	yes	279.4304	alive	711	no
62	TCGA-DJ-A13V	21	F	no	total	Lt	yes	2.9		8	20	T3	N1a	M0	I	yes	165.1811	alive	1313	no
63	TCGA-DJ-A1QD	20	F	no	total	Rt	no	2.2		12	30	T2	N1b	M0	I	yes	267.8416	alive	335	no
64	TCGA-DJ-A1QE	62	F	no	Lobectomy	Rt	yes	1.5		0	0	T3	Nx	M0	III	yes	297.1238	alive	4134	no
65	TCGA-DJ-A1QF	61	F	no	total	Bilat	no	2.9		0	0	T2	Nx	M0	II	yes	324.3667	alive	931	no

66	TCGA-DJ-A1QI	63	F	no	total	Bilat	no	3	0	0	T2	Nx	M0	II	yes	232.1991	alive	455	no
67	TCGA-DJ-A1QN	42	F	no	total	Bilat	no	1.5	0	0	T1b	Nx	M0	I	yes	219.1634	alive	1295	no
68	TCGA-DJ-A1QQ	43	M	no	total	Bilat	yes	3	0	1	T3	N0	M0	I	yes	257.1532	alive	534	no
69	TCGA-DJ-A2PN	70	F	no	total	Lt	no	1.3	0	0	T1b	Nx	M0	I	yes	201.1418	alive	307	no
70	TCGA-DJ-A2PO	54	M	no	total	Bilat	no	0.6	0	5	T1a	N0	M0	I	yes	216.2453	alive	243	no
71	TCGA-DJ-A2PQ	26	M	no	total	Lt	no	3.5	7	12	T2	N1a	M0	I	yes	189.7891	alive	1077	no
72	TCGA-DJ-A2PR	27	M	yes	total	Bilat	yes	2	6	12	T3	N1a	M0	I	yes	149.4736	alive	835	no
73	TCGA-DJ-A2PS	40	F	no	total	Bilat	yes	1.1	1	4	T3	N1b	M0	I	yes	169.9574	alive	797	no
74	TCGA-DJ-A2PU	52	F	no	total	Bilat	no	2.8	0	3	T2	N0	M0	II	yes	164.8631	alive	382	no
75	TCGA-DJ-A2PV	53	F	no	total	Bilat	no	3.4	0	6	T2	N0	M0	II	yes	212.853	alive	252	no
76	TCGA-DJ-A2PW	65	M	no	total	Rt+Isthmus	no	4.6	1	1	T3	N1a	M0	III	yes	125.2512	alive	1060	no
77	TCGA-DJ-A2PZ	63	M	no	Lobectomy	Lt+Isthums	yes	1.7	0	1	T3	N0	M0	III	yes	182.4077	alive	256	no
78	TCGA-DJ-A2Q0	57	F	no	Lobectomy	Rt+Isthmus	no	2.8	0	0	T2	Nx	M0	II	no	313.8253	alive	1045	no
79	TCGA-DJ-A2Q1	44	F	no	total	Bilat	no	1.6	11	44	T1b	N1b	M0	I	no	45.2991	alive	1148	no
80	TCGA-DJ-A2Q4	53	M	no	total	Bilat	no	1.3	1	10	T1b	N1a	M0	III	yes	187.0691	alive	287	no
81	TCGA-DJ-A2Q5	51	M	no	total	Lt	no	4	2	5	T2	N1b	M0	IV	yes	158.4914	alive	1140	no
82	TCGA-DJ-A2Q6	38	F	no	total	Bilat	yes	1.1	22	45	T3	N1b	M0	I	yes	196.119	alive	1109	no
83	TCGA-DJ-A2Q7	52	F	no	total	Bilat	yes	2.5	3	5	T3	N1b	M0	IV	yes	147.8172	alive	307	no
84	TCGA-DJ-A2QC	70	F	no	total	Lt	no	2.3	0	0	T2	Nx	M0	II	yes	100.0426	alive	483	no
85	TCGA-DJ-A3UK	35	F	no	total	Lt	no	1.5	0	2	T1b	N0	M0	I	yes	186.2911	alive	459	no
86	TCGA-DJ-A3UM	45	F	no	total	Lt	no	1.3	0	3	T1b	N0	M0	I	yes	225.0223	alive	944	no
87	TCGA-DJ-A3UN	51	F	no	total	Bilat	no	1.2	0	3	T1b	N0	M0	I	yes	216.0674	alive	622	no
88	TCGA-DJ-A3UO	63	M	no	total	Bilat	yes	2.8	6	25	T3	N1b	M0	IV	yes	210.1999	alive	957	yes

89	TCGA-DJ-A3US	37	F	no	total	Rt	no	1.5	0	0	T1b	Nx	M0	I	no	168.5533	alive	998	no
90	TCGA-DJ-A3UU	49	F	no	total	Bilat	yes	1.1	0	2	T3	N0	M0	III	yes	201.3498	alive	538	no
91	TCGA-DJ-A3UW	50	F	no	total	Bilat	no	1.2	0	4	T1b	N0	M0	I	yes	154.5415	alive	1812	no
92	TCGA-DJ-A3UX	46	F	no	total	Rt	no	0.5	0	3	T1a	N0	M0	I	yes	232.7641	alive	345	no
93	TCGA-DJ-A3UY	32	F	no	total	Lt	no	1.2	0	0	T1b	N0	M0	I	yes	250.1771	alive	650	no
94	TCGA-DJ-A3UZ	70	F	yes	total	Bilat	yes	1.1	0	2	T3	N0	M0	III	no	173.3423	alive	563	no
95	TCGA-DJ-A3V2	44	F	no	total	Bilat	no	2.1	1	4	T2	N1a	M0	I	no	138.684	alive	615	no
96	TCGA-DJ-A3V3	57	F	no	total	Lt	no	2.4	0	4	T2	N0	M0	II	no	104.5372	alive	931	no
97	TCGA-DJ-A3V9	50	F	no	total	Bilat	no	2.3	12	56	T2	N1b	M0	IV	no	157.803	alive	623	no
98	TCGA-DJ-A3VA	39	F	no	total	Lt+Isthums	no	2	1	28	T1b	N1a	M0	I	yes	253.93	alive	755	no
99	TCGA-DJ-A3VD	32	F	no	total	Isthmus	no	0.8	6	14	T1a	N1a	M0	I	no	198.146	alive	639	no
100	TCGA-DJ-A3VE	50	M	no	total	Lt	no	1.7	0	0	T1b	Nx	M0	I	yes	154.8214	alive	577	no
101	TCGA-DJ-A4UL	68	F	no	total	Bilat	no	1.1	0	3	T1b	N0	M0	I	yes	105.8294	alive	554	no
102	TCGA-DJ-A4UP	15	F	no	total	Rt	no	1.6	5	49	T1b	N1b	M0	I	no	107.6741	alive	649	no
103	TCGA-DJ-A4UT	32	F	no	total	Bilat	no	1	0	0	T1a	Nx	M0	I	no	136.1111	alive	430	no
104	TCGA-DJ-A4V0	36	F	no	total	Lt	no	2.1	0	0	T2	Nx	M0	I	no	156.4225	alive	542	no
105	TCGA-DJ-A4V2	38	F	no	total	Rt	no	3	0	1	T2	N0	M0	I	yes	209.1619	alive	95	no
106	TCGA-DJ-A4V5	55	M	no	total	Rt	yes	4.1	12	13	T3	N1b	M0	IV	no	142.0421	alive	740	no
107	TCGA-DO-A1K0	30	F	no	total	Bilat	yes	4.2	8	14	T3	N1b	MX	I	yes	321.0526	alive	655	yes
108	TCGA-DO-A2HM	49	M	n.a	total	Lt	no	1.8	3	5	T1b	N1b	MX	IV	yes	150.8907	alive	71	no
109	TCGA-E3-A3E0	34	F	no	total	Lt	no	2.4	0	3	T2	N0	MX	I	no	190.232	alive	2019	no
110	TCGA-E3-A3E1	46	F	n.a	Lobectomy	Rt	no	1.5	0	2	T1b	N0	M0	I	yes	247.4444	alive	1223	no
111	TCGA-E3-A3E3	49	F	no	total	Rt	no	2.8	0	1	T2	N0	M0	II	yes	243.5323	alive	922	no

112	TCGA-E3-A3E5	57	M	no	total	Bilat	yes	1		13	28	T4a	N1b	MX	IV	yes	197.3836	alive	427	no
113	TCGA-E8-A242	56	F	no	total	Lt+Isthums	yes	1.8		0	1	T3	N0	MX	III	yes	167.2834	alive	950	no
114	TCGA-E8-A2EA	52	F	no	Subtotal	Rt	no	1.5	n.a	n.a		T1b	Nx	M0	I	yes	114.1173	alive	678	no
115	TCGA-E8-A2JQ	18	F	no	Unreported	Rt	yes	2.1		11	14	T3	N1a	M0	I	no	147.656	alive	356	no
116	TCGA-E8-A3X7	57	F	no	Unreported	Rt	yes	1	n.a	n.a		T3	Nx	M0	III	no	154.8673	alive	596	no
117	TCGA-E8-A413	38	F	no	Unreported	Lt	no	1.5	n.a	n.a		T1b	Nx	M0	I	yes	130.7475	alive	414	no
118	TCGA-E8-A414	45	F	no	Unreported	Lt	no	5	n.a	n.a		T3	Nx	M0	III	no	203.6446	alive	434	no
119	TCGA-E8-A415	39	F	n.a	Unreported	Lt	no	1	n.a	n.a		T1a	Nx	M0	I	yes	192.5715	alive	420	no
120	TCGA-E8-A416	51	F	n.a	Unreported	Lt	no	1.5	n.a	n.a		T1b	Nx	M0	I	no	188.239	alive	428	no
121	TCGA-E8-A417	38	F	n.a	Unreported	Rt	no	2.5	n.a	n.a		T2	Nx	M0	I	no	271.3374	alive	434	no
122	TCGA-E8-A418	75	F	no	Unreported	Rt	yes	9	n.a	n.a		T3	Nx	M0	III	yes	264.553	alive	92	no
123	TCGA-E8-A419	30	F	no	Unreported	Lt	no	3	n.a	n.a		T2	Nx	M0	I	yes	165.6051	alive	774	no
124	TCGA-E8-A432	57	F	no	Unreported	Lt	no	2.5	n.a	n.a		T2	Nx	M0	II	no	104.4202	alive	439	no
125	TCGA-E8-A433	25	F	no	Unreported	Rt	no	2.5	n.a	n.a		T2	Nx	M0	I	yes	139.9107	alive	447	no
126	TCGA-E8-A434	56	F	no	Unreported	Lt	no	0.7		0	0	T1a	N0	M0	I	no	151.4664	alive	429	no
127	TCGA-E8-A436	53	F	n.a	Unreported	Bilat	no	2.5	n.a	n.a		T2	N0	M0	II	yes	177.1013	alive	455	no
128	TCGA-E8-A437	57	F	no	Lobectomy	Rt	no	1.5		0	0	T1b	Nx	M0	I	yes	131.9149	alive	234	no
129	TCGA-E8-A438	23	F	no	Lobectomy	Lt	no	2.3		0	3	T2	N0	M0	I	no	76.1082	alive	629	no
130	TCGA-E8-A44K	50	F	no	Unreported	Lt	no	2	n.a	n.a		T1b	Nx	M0	I	yes	191.4624	alive	419	no
131	TCGA-E8-A44M	23	F	no	Unreported	Rt	no	1	n.a	n.a		T1a	Nx	M0	I	no	107.7712	alive	413	no
132	TCGA-EL-A3CL	70	F	no	total	Isthmus	no	3		0	0	T2	Nx	M0	II	yes	201.9614	dead	963	no
133	TCGA-EL-A3CM	64	F	n.a	total	Bilat	yes	5		2	16	T4a	N1b	M0	IV	yes	264.9713	dead	813	no
134	TCGA-EL-A3CN	47	F	no	total	Lt	yes	1.5		0	9	T3	N0	M0	III	yes	196.8332	alive	4731	no

135	TCGA-EL-A3CO	88	M	no	total	Bilat	yes	3.5	0	0	T3	N1b	M0	IV	no	195.3665	dead	2973	no
136	TCGA-EL-A3CR	78	F	no	total	Rt	yes	3.5	8	32	T3	N1b	M0	IV	yes	108.127	dead	233	no
137	TCGA-EL-A3CS	62	F	yes	Completion	Rt	yes	4	0	20	T4a	N0	M0	IV	yes	328.473	dead	1385	no
138	TCGA-EL-A3CT	78	F	no	total	Bilat	yes	4	2	17	T4a	N1b	M0	IV	yes	377.994	dead	1854	no
139	TCGA-EL-A3CU	70	F	no	total	Rt	no	1.5	4	7	T1b	N1a	M0	III	yes	231.5338	alive	4780	no
140	TCGA-EL-A3CV	40	M	no	total	Bilat	yes	4	18	105	T3	N1b	M0	I	yes	323.7521	alive	1154	no
141	TCGA-EL-A3CW	87	F	no	Lobectomy	Rt+Isthmus	no	3.5	14	21	T2	N1b	M0	IV	yes	143.0064	dead	1734	no
142	TCGA-EL-A3CX	22	F	no	total	Rt	no	3.3	0	13	T2	N0	M0	I	no	80.303	alive	2461	no
143	TCGA-EL-A3CY	28	M	yes	completion	Rt	yes	5	12	12	T3	N1b	M0	I	no	161.0852	alive	127	no
144	TCGA-EL-A3CZ	37	F	no	total	Lt	no	1	1	7	T1a	N1a	M0	I	no	251.5576	alive	1789	yes
145	TCGA-EL-A3D0	61	M	no	total	Lt	yes	4.8	9	37	T3	N1b	M0	IV	yes	158.4621	alive	4254	no
146	TCGA-EL-A3D1	39	M	no	total	Lt	no	3	0	19	T2	N0	M0	I	yes	87.033	alive	2997	no
147	TCGA-EL-A3D4	62	M	no	total	Bilat	yes	4	31	75	T3	N1b	M0	IV	no	100.4367	alive	1381	no
148	TCGA-EL-A3D5	44	F	yes	total	Bilat	yes	2.5	16	31	T3	N1b	M0	I	no	249.0827	alive	1737	no
149	TCGA-EL-A3D6	51	F	no	total	Lt	yes	5.5	1	6	T4a	N1b	M0	IV	yes	194.3684	alive	3296	no
150	TCGA-EL-A3GP	70	M	no	total	Rt	yes	5	7	15	T3	N1b	M0	IV	yes	192.2197	alive	43	no
151	TCGA-EL-A3GR	31	F	no	total	Rt	yes	3	15	20	T3	N1a	M0	I	yes	268.5074	alive	3501	no
152	TCGA-EL-A3GS	37	F	no	total	Bilat	yes	1.6	1	1	T3	N1a	M0	I	yes	277.2895	alive	1989	no
153	TCGA-EL-A3GU	72	F	no	total	Bilat	yes	5	10	23	T3	N1b	M0	IV	yes	217.9648	alive	4137	yes
154	TCGA-EL-A3GV	65	F	no	total	Lt	yes	1.9	0	1	T3	N0	M0	III	yes	247.0276	alive	1532	no
155	TCGA-EL-A3GW	37	F	no	total	Rt	no	2.5	0	0	T2	Nx	M0	I	no	224.5073	alive	1527	no
156	TCGA-EL-A3GX	41	F	no	total	Bilat	no	2.5	2	21	T2	N1b	M0	I	yes	224.9407	alive	4494	yes
157	TCGA-EL-A3GY	40	F	no	total	Rt	no	1.5	1	2	T1b	N1a	M1	II	yes	244.2748	alive	3076	no

158	TCGA-EL-A3GZ	34	F	no	total	Lt	no	1.8	0	4	T1b	N0	M0	I	yes	162.726	alive	4258	no
159	TCGA-EL-A3H1	66	F	n.a	total	Rt	no	2	0	0	T1b	Nx	M0	I	no	186.3272	dead	1500	no
160	TCGA-EL-A3H2	58	M	yes	total	Bilat	no	0.3	4	22	T1a	N1b	MX	IV	no	148.0098	dead	1019	no
161	TCGA-EL-A3H3	19	F	no	total	Isthmus	no	2.7	6	19	T2	N1b	M0	I	no	157.9577	alive	1319	no
162	TCGA-EL-A3H4	46	F	no	total	Rt	yes	5	8	71	T3	N1b	M0	IV	yes	400.8592	alive	1512	no
163	TCGA-EL-A3H5	57	F	no	total	Bilat	yes	1.8	6	48	T4a	N1b	M0	IV	yes	169.4215	alive	2542	no
164	TCGA-EL-A3H7	36	F	no	total	Bilat	yes	3	8	9	T3	N1a	M0	I	yes	196.4716	alive	4228	no
165	TCGA-EL-A3H8	35	F	no	total	Bilat	yes	2	17	48	T3	N1b	M0	I	yes	331.4873	alive	3989	no
166	TCGA-EL-A3MW	55	F	no	total	Rt	no	1.5	0	1	T1b	N0	M0	I	yes	185.0917	alive	3305	no
167	TCGA-EL-A3MX	66	F	no	total	Lt	no	5	0	2	T3	N0	M1	IV	yes	95.0168	dead	1753	yes
168	TCGA-EL-A3MY	81	M	no	total	Lt	yes	1.5	0	0	T3	Nx	M1	IV	yes	178.8941	dead	533	no
169	TCGA-EL-A3MZ	66	M	no	total	Lt+Isthums	yes	3.6	1	2	T4a	N1a	MX	IV	yes	249.0092	dead	1597	no
170	TCGA-EL-A3N2	24	F	no	total	Lt	yes	3.5	5	27	T3	N1b	M0	I	yes	253.8386	alive	902	no
171	TCGA-EL-A3N3	53	F	no	total	Lt	yes	1.5	0	0	T3	Nx	M0	III	yes	225.2101	alive	4484	no
172	TCGA-EL-A3T0	45	F	no	total	Bilat	yes	2	10	31	T3	N1b	M0	IV	no	310.0856	alive	3976	no
173	TCGA-EL-A3T1	38	F	no	total	Isthmus	yes	3.8	0	1	T3	N0	M0	I	yes	239.0234	alive	3941	no
174	TCGA-EL-A3T2	55	F	no	total	Rt	no	1	3	15	T1a	N1b	M0	IV	no	280.5663	alive	988	yes
175	TCGA-EL-A3T3	63	M	no	total	Rt	no	3.5	0	0	T2	Nx	M0	II	yes	140.4604	alive	4017	no
176	TCGA-EL-A3T6	34	F	no	total	Lt	no	4.5	0	1	T3	N0	M0	I	yes	205.7354	alive	2988	no
177	TCGA-EL-A3T7	47	F	no	total	Rt	yes	2	0	0	T3	Nx	M0	III	yes	157.9341	alive	1256	no
178	TCGA-EL-A3T8	36	M	no	total	Bilat	no	3.8	0	0	T2	Nx	M0	I	yes	201.029	alive	3269	no
179	TCGA-EL-A3T9	69	F	no	total	Lt	yes	4	2	2	T4a	N1a	MX	IV	no	175.253	dead	174	no
180	TCGA-EL-A3TA	42	M	no	total	Lt	no	3.5	0	15	T2	N0	M0	I	yes	131.6297	alive	2035	no

181	TCGA-EL-A3TB	47	F	no	total	Rt	yes	2.2	15	29	T3	N1b	M0	IV	no	122.7935	alive	2519	no
182	TCGA-EL-A3ZG	15	M	no	total	Lt	no	6	0	1	T3	N0	M0	I	no	198.1634	alive	2724	no
183	TCGA-EL-A3ZH	42	F	no	total	Lt	yes	2.5	4	8	T3	N1a	M0	I	no	211.3438	alive	2402	no
184	TCGA-EL-A3ZK	41	F	no	total	Rt	no	2.5	3	17	T2	N1b	M0	I	no	459.306	alive	958	no
185	TCGA-EL-A3ZL	34	F	no	total	Rt	no	1.5	7	25	T1b	N1b	M0	I	no	160.4783	alive	1895	no
186	TCGA-EL-A3ZM	60	M	no	total	Rt+Isthmus	yes	5.7	7	26	T4b	N1b	M0	IV	no	129.5959	alive	1096	no
187	TCGA-EL-A3ZN	28	F	no	total	Lt	yes	1.3	6	55	T3	N1b	M0	I	no	108.3333	alive	1614	no
188	TCGA-EL-A3ZO	79	F	no	total	Rt	yes	2.4	1	7	T3	N1a	M0	III	no	86.435	alive	1441	no
189	TCGA-EL-A3ZP	19	M	no	total	Rt	yes	2.6	9	50	T3	N1b	M0	I	no	123.6623	alive	1597	no
190	TCGA-EL-A3ZQ	78	F	no	total	Rt	no	3.6	0	1	T2	N0	M0	II	yes	60.94	alive	1862	no
191	TCGA-EL-A3ZR	46	F	no	Lobectomy	Lt	no	2.2	0	0	T2	Nx	M0	II	no	115.3025	alive	1830	no
192	TCGA-EL-A3ZS	22	F	no	total	Isthmus	no	1.6	13	40	T1b	N1b	M0	I	no	191.3423	alive	215	no
193	TCGA-EL-A3ZT	35	M	no	total	Lt	yes	2.5	0	0	T3	Nx	M0	I	yes	148.7265	alive	1808	no
194	TCGA-EL-A4JV	41	F	no	Lobectomy	Rt	no	2.5	0	1	T2	N0	M0	I	no	166.6269	alive	1748	no
195	TCGA-EL-A4JW	38	F	no	total	Rt	no	1.6	0	1	T1b	N0	M0	I	yes	186.271	alive	1056	no
196	TCGA-EL-A4JX	31	F	no	total	Rt	no	4	0	1	T3	N0	MX	I	yes	160.9823	alive	1398	no
197	TCGA-EL-A4JZ	55	F	no	total	Isthmus	yes	2	6	25	T3	N1b	M0	IV	yes	104.4788	alive	1154	no
198	TCGA-EL-A4K0	54	F	no	total	Rt	no	1.1	0	1	T1b	N0	M0	I	yes	157.5342	alive	944	no
199	TCGA-EL-A4K1	68	F	no	total	Rt	yes	2.9	3	5	T3	N1a	M0	III	no	178.606	alive	1046	no
200	TCGA-EL-A4K2	43	F	no	Lobectomy	Lt	no	1.8	0	1	T1b	N0	M0	I	no	131.182	alive	928	no
201	TCGA-EL-A4K4	33	F	no	total	Lt	yes	4.4	13	43	T4a	N1b	M0	I	yes	285.7326	alive	1623	no
202	TCGA-EL-A4K6	75	M	no	total	Rt	yes	6.2	3	42	T3	N1a	MX	III	no	148.8424	alive	1343	yes
203	TCGA-EL-A4K7	74	M	no	total	Lt	yes	2.5	1	1	T3	N1a	M0	III	no	86.4417	alive	1251	no

204	TCGA-EL-A4K9	68	M	no	total	Rt	yes	1.5	0	0	T3	Nx	M0	III	no	142.6102	alive	1455	no
205	TCGA-EL-A4KD	41	M	no	total	Lt	yes	4.5	4	18	T3	N1a	M0	I	no	125.4061	alive	1326	no
206	TCGA-EL-A4KG	35	F	no	total	Rt	yes	2	0	1	T3	N0	M0	I	yes	134.7518	alive	739	no
207	TCGA-EL-A4KH	36	F	no	total	Lt	no	2.1	0	0	T2	Nx	M0	I	yes	61.7056	alive	823	no
208	TCGA-EL-A4KI	63	M	no	total	Bilat	yes	3.4	0	0	T3	N0	MX	III	no	64.7826	alive	727	yes
209	TCGA-EM-A1CS	55	F	n.a	total	Bilat	no	1.3	0	6	T1b	N0	MX	I	no	228.0188	alive	817	no
210	TCGA-EM-A1CT	76	M	n.a	total	Rt	no	1.5	1	22	T1b	N1b	MX	IV	yes	255.168	alive	553	no
211	TCGA-EM-A1CU	31	M	n.a	total	Bilat	no	6.5	1	20	T3	N1b	M0	I	yes	177.3436	alive	636	no
212	TCGA-EM-A1CV	32	F	n.a	total	Bilat	no	1.1	0	3	T1b	N0	MX	I	yes	256.816	alive	376	no
213	TCGA-EM-A22I	52	F	no	total	Bilat	yes	2.1	0	1	T3	N0	MX	III	yes	221.968	alive	448	no
214	TCGA-EM-A22K	46	F	no	total	Bilat	no	1.1	10	83	T1b	N1b	MX	IV	no	193.6994	alive	479	no
215	TCGA-EM-A22M	52	M	no	total	Bilat	no	1.7	0	3	T1b	N0	MX	I	yes	114.4468	alive	442	no
216	TCGA-EM-A22O	75	M	no	total	Bilat	yes	6.6	1	1	T4a	N1a	MX	IV	yes	276.5336	alive	357	no
217	TCGA-EM-A22P	64	M	no	total	Bilat	no	1.2	14	28	T1b	N1b	MX	IV	yes	154.3134	alive	339	no
218	TCGA-EM-A2CS	51	F	no	total	Rt	no	6.3	35	49	T3	N1b	MX	IV	no	257.308	alive	841	no
219	TCGA-EM-A2OX	46	M	no	total	Rt	no	1.8	8	9	T1b	N1b	MX	IV	yes	152.4993	alive	600	no
220	TCGA-EM-A2OZ	66	M	yes	total	Lt+Isthums	no	1.7	0	2	T1b	N0	MX	I	yes	267.9277	alive	1033	no
221	TCGA-EM-A2P0	33	M	no	total	Lt	no	2.1	9	34	T2	N1b	MX	I	yes	232.687	alive	535	no
222	TCGA-EM-A2P1	33	M	no	total	Lt	no	3.1	15	54	T2	N1b	MX	I	yes	252.9766	alive	531	yes
223	TCGA-EM-A2P3	47	F	no	Lobectomy	Rt	no	1.4	0	0	T1b	Nx	MX	I	yes	236.9635	alive	444	no
224	TCGA-EM-A3AK	62	F	no	total	Bilat	no	2	0	2	T1b	N0	MX	I	yes	176.4008	alive	477	no
225	TCGA-EM-A3AN	36	F	no	total	Rt	no	1.2	27	80	T1b	N1b	MX	I	no	144.0708	alive	1006	no
226	TCGA-EM-A3AO	61	M	no	total	Bilat	no	3	10	26	T2	N1b	MX	IV	no	70.6483	alive	407	no

227	TCGA-EM-A3AQ	85	F	no	total	Bilat	no	1.3	0	3	T1b	N0	MX	I	no	118.0279	alive	904	no
228	TCGA-EM-A3AR	41	M	no	total	Bilat	no	1.2	1	1	T1b	N1a	MX	I	yes	164.7963	alive	1133	no
229	TCGA-EM-A3FJ	24	F	no	total	Rt	no	1	12	33	T1a	N1b	MX	I	yes	125.4431	alive	850	no
230	TCGA-EM-A3FK	30	F	no	total	Bilat	no	1.2	17	25	T1b	N1b	MX	I	yes	189.7473	alive	723	no
231	TCGA-EM-A3FM	57	M	no	total	Bilat	no	2.2	17	53	T2	N1b	MX	IV	yes	197.5934	alive	639	yes
232	TCGA-EM-A3FO	40	M	no	total	Bilat	no	4.4	0	3	T3	N0	MX	I	yes	134.9333	alive	532	no
233	TCGA-EM-A3FR	55	F	no	total	Bilat	no	3	2	7	T2	N1a	MX	III	no	166.5362	alive	457	no
234	TCGA-EM-A3O3	83	F	no	total	Rt+Isthmus	no	3.1	3	16	T2	N1a	MX	III	yes	56.1447	alive	441	no
235	TCGA-EM-A3O7	46	F	no	total	Rt+Isthmus	no	5	0	0	T3	Nx	MX	III	yes	115.8537	alive	223	no
236	TCGA-EM-A3SU	43	F	no	total	Bilat	no	2.1	3	33	T2	N1b	MX	I	no	171.8377	alive	911	no
237	TCGA-EM-A3SX	28	F	no	total	Bilat	no	4.4	3	4	T3	N1a	MX	I	no	174.9221	alive	475	no
238	TCGA-EM-A3SZ	42	F	no	total	Bilat	no	2.6	0	4	T2	N0	MX	I	no	185.3491	alive	389	no
239	TCGA-EM-A4FF	40	F	no	total	Lt	no	1.5	1	4	T1b	N1a	MX	I	no	216.7147	alive	2573	yes
240	TCGA-EM-A4FM	59	F	no	total	Bilat	yes	3	12	40	T3	N1b	MX	IV	yes	163.4262	alive	162	no
241	TCGA-EM-A4FN	49	F	no	total	Bilat	no	1.9	8	32	T1b	N1b	MX	IV	no	147.7249	alive	802	no
242	TCGA-EM-A4FO	72	M	no	total	Bilat	no	1.1	0	6	T1b	N0	MX	I	yes	111.8304	alive	802	no
243	TCGA-EM-A4FV	64	F	no	total	Lt	no	1.3	0	5	T1b	N0	MX	I	yes	154.6794	alive	744	no
244	TCGA-EM-A4G1	26	F	no	total	Lt	no	1.7	0	2	T1b	N0	MX	I	no	238.5321	alive	416	no
245	TCGA-ET-A25J	40	F	no	total	Rt	no	2.5	2	18	T2	N1a	MX	I	yes	266.9527	alive	903	no
246	TCGA-ET-A25K	36	F	no	total	Bilat	no	2.5	3	11	T2	N1a	MX	I	yes	201.1555	alive	984	no
247	TCGA-ET-A25M	33	M	no	total	Rt	no	2.5	1	2	T2	N1a	MX	I	no	271.9251	alive	375	no
248	TCGA-ET-A25N	28	F	no	total	Rt	no	2.3	1	8	T2	N1a	MX	I	no	265.1167	alive	450	no
249	TCGA-ET-A25O	35	F	no	total	Lt	no	2.3	2	5	T2	N1a	MX	I	yes	289.0165	alive	824	no

250	TCGA-ET-A25P	24	F	no	total	Lt	no	0.7	0	3	T1a	N0	MX	I	no	138.6169	alive	559	no
251	TCGA-ET-A2MX	27	M	n.a	total	Rt	no	2.1	4	4	T2	N1a	MX	I	no	175.0834	alive	1644	no
252	TCGA-ET-A2MY	72	F	no	total	Rt+Isthmus	no	1.3	0	0	T1b	Nx	MX	I	yes	215.3732	alive	672	no
253	TCGA-ET-A2MZ	39	M	no	total	Rt	no	1.2	0	6	T1b	N0	MX	I	yes	245.1278	alive	373	no
254	TCGA-ET-A2N0	51	F	no	total	Rt	no	2	20	27	T1b	N1a	MX	III	yes	170	alive	489	no
255	TCGA-ET-A39J	23	F	no	total	Bilat	no	3	0	0	T2	Nx	MX	I	yes	171.8369	alive	2160	no
256	TCGA-ET-A39K	48	F	no	total	Lt	yes	4	11	14	T3	N1a	MX	III	yes	84.1232	alive	2434	yes
257	TCGA-ET-A39L	20	F	no	total	Lt	no	5.5	0	4	T3	N0	MX	I	no	179.704	alive	1988	no
258	TCGA-ET-A39M	34	M	yes	total	Lt	no	4	0	0	T2	Nx	MX	I	yes	74.0466	alive	2582	no
259	TCGA-ET-A39N	35	F	no	total	Rt	no	5	0	0	T3	Nx	MX	I	no	369.13	alive	2998	no
260	TCGA-ET-A39P	73	F	no	total	Lt	yes	5.3	0	2	T3	N0	MX	III	yes	187.353	alive	1786	no
261	TCGA-ET-A39R	24	F	no	total	Bilat	no	1.8	0	18	T1b	N0	MX	I	no	132.3897	alive	1737	no
262	TCGA-ET-A39S	27	F	no	total	Lt	no	1	0	2	T1a	N0	MX	I	yes	150.2161	alive	1676	no
263	TCGA-ET-A3BN	33	F	no	total	Rt	no	3	0	0	T2	Nx	MX	I	no	314.6949	alive	859	no
264	TCGA-ET-A3BP	34	F	no	total	Lt+Isthums	no	2.5	2	3	T2	N1a	MX	I	yes	134.323	alive	127	no
265	TCGA-ET-A3BQ	27	F	no	total	Rt	no	3	0	2	T2	N0	MX	I	yes	179.0195	alive	74	no
266	TCGA-ET-A3BS	42	M	yes	total	Bilat	yes	5.5	8	17	T3	N1a	MX	I	yes	186.446	alive	169	no
267	TCGA-ET-A3BU	28	M	no	total	Bilat	no	1.4	6	9	T1b	N1a	MX	I	yes	199.3176	alive	1165	no
268	TCGA-ET-A3BV	47	F	no	total	Lt	no	4	2	17	T2	N1a	MX	III	yes	180.3412	alive	1285	yes
269	TCGA-ET-A3BW	30	F	no	total	Lt	no	3.2	0	0	T2	N0	MX	I	yes	246.6527	alive	72	no
270	TCGA-ET-A3BX	24	M	no	total	Lt+Isthums	yes	5	1	4	T3	N1a	MX	I	yes	208.1612	alive	137	no
271	TCGA-ET-A3DO	33	F	no	total	Bilat	no	2.5	0	0	T2	Nx	MX	I	yes	159.1956	alive	2458	yes
272	TCGA-ET-A3DP	43	F	no	total	Lt	no	2.5	0	0	T2	Nx	MX	I	yes	260.1913	alive	2106	no

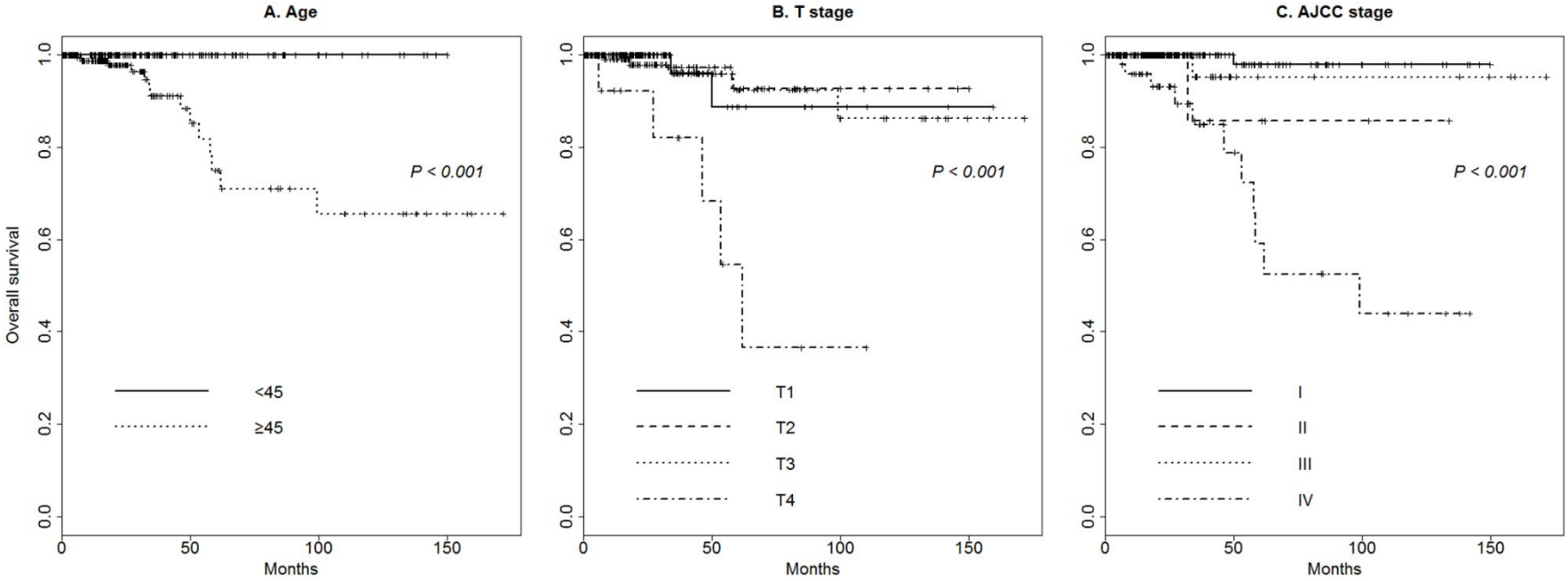
273	TCGA-ET-A3DR	42	F	no	total	Lt	no	1.2	5	6	T1b	N1a	MX	I	no	196.4912	alive	1323	no
274	TCGA-ET-A3DS	33	F	no	total	Rt	no	0.8	0	8	T1a	N0	MX	I	no	193.5364	alive	9	no
275	TCGA-ET-A3DT	23	F	no	total	Lt	no	2.5	1	4	T2	N1a	MX	I	yes	202.381	alive	547	no
276	TCGA-ET-A3DU	21	F	no	Lobectomy	Lt	no	1.4	1	2	T1b	N1a	MX	I	yes	182.5059	alive	770	no
277	TCGA-ET-A40S	62	M	yes	total	Bilat	no	2.5	0	12	T2	N0	MX	II	no	100.4301	alive	1223	no
278	TCGA-ET-A40T	26	F	no	total	Lt+Isthums	no	2.8	3	15	T2	N1a	MX	I	no	118.1919	alive	1140	no
279	TCGA-FE-A230	30	F	no	total	Lt	no	4.4	3	3	T3	N1a	MX	I	yes	285.2495	alive	1816	yes
280	TCGA-FE-A231	70	M	no	total	Rt	no	9.4	16	31	T3	N1b	MX	IV	yes	310.9322	alive	961	no
281	TCGA-FE-A232	44	F	no	total	Rt	yes	3.5	0	0	T3	Nx	MX	I	yes	194.1419	alive	1346	no
282	TCGA-FE-A233	18	F	no	total	Rt	no	2.3	0	0	T2	Nx	MX	I	yes	348.2024	alive	751	yes
283	TCGA-FE-A234	26	F	no	total	Rt	no	3.3	2	2	T2	N1a	MX	I	yes	263.187	alive	2075	yes
284	TCGA-FE-A235	26	F	no	total	Bilat	no	3	3	4	T2	N1a	MX	I	yes	104.3992	alive	3571	no
285	TCGA-FE-A236	33	M	no	total	Lt	no	3.5	5	5	T2	N1a	MX	I	yes	297.8541	alive	2031	no
286	TCGA-FE-A237	19	F	no	Lobectomy	Rt	yes	3.5	1	1	T3	N1a	MX	I	yes	251.238	alive	2474	yes
287	TCGA-FE-A238	36	F	no	total	Rt	yes	3.9	0	1	T3	N0	MX	I	no	239.241	alive	1215	yes
288	TCGA-FE-A239	82	M	no	total	Rt	no	4.5	0	2	T3	N0	MX	III	no	165.81	dead	1023	yes
289	TCGA-FE-A23A	27	F	no	total	Rt	no	2.7	0	13	T2	N0	MX	I	yes	192.2054	alive	2489	no
290	TCGA-FE-A3PB	33	F	no	total	Rt	no	1	5	6	T1a	N1b	MX	I	yes	151.1185	alive	2590	no
291	TCGA-FE-A3PC	37	F	no	total	Lt	no	5.2	19	19	T3	N1a	MX	I	yes	114.9279	alive	2606	no
292	TCGA-FK-A3SB	28	F	no	total	Rt	yes	2	2	14	T3	N1a	MX	I	yes	173.7047	alive	125	no
293	TCGA-FK-A3SD	61	F	no	completion	Lt	no	0.1	0	0	T1a	Nx	M0	I	no	128.9123	alive	32	no
294	TCGA-FK-A3SE	31	F	no	total	Bilat	no	3.5	4	15	T2	N1a	MX	I	no	229.2445	alive	670	no
295	TCGA-FK-A3SG	21	F	no	total	Rt	no	3.2	11	27	T2	N1b	MX	I	no	159.9223	alive	28	no

296	TCGA-FK-A3SH	50	F	no	total	Bilat	no	3	4	12	T2	N1a	MX	III	yes	75.5782	alive	1463	no
297	TCGA-FK-A4UB	51	M	no	total	Rt	yes	2	19	98	T3	N1b	MX	IV	no	110.7804	alive	3531	no
298	TCGA-FY-A2QD	61	F	n.a	total	Lt	no	0.6	0	3	T1a	N0	MX	I	no	194.1564	alive	38	no
299	TCGA-FY-A3BL	29	M	n.a	Lobectomy	Rt	no	1.2	0	0	T1b	Nx	M0	I	yes	258.1804	alive	621	no
300	TCGA-FY-A3I4	34	F	n.a	total	Rt	no	4	0	1	T2	N0	M0	I	yes	168.4266	alive	542	no
301	TCGA-FY-A3NM	48	F	no	total	Bilat	yes	1.6	0	6	T3	N0	MX	III	no	314.9242	alive	30	no
302	TCGA-FY-A3NN	48	F	n.a	total	Rt	no	2	2	2	T1b	N1a	MX	III	yes	274.7385	alive	1	no
303	TCGA-FY-A3ON	20	M	no	total	Bilat	no	2.5	1	4	T2	N1a	MX	I	yes	167.863	alive	595	no
304	TCGA-FY-A3R6	57	F	n.a	total	Lt	no	2.2	5	30	T2	N1b	MX	IV	no	133.8028	alive	423	no
305	TCGA-FY-A3R7	50	F	no	total	Bilat	yes	2	2	2	T3	N1a	MX	III	yes	201.9584	alive	495	no
306	TCGA-FY-A3R8	62	F	n.a	total	Lt	yes	1.7	0	0	T3	Nx	MX	III	yes	192.3566	alive	477	no
307	TCGA-FY-A3R9	66	F	n.a	total	Bilat	no	0.7	0	1	T1a	N0	MX	I	no	188.4439	alive	504	no
308	TCGA-FY-A3RA	21	F	n.a	total	Lt	no	4	0	0	T2	Nx	MX	I	yes	251.5901	alive	550	no
309	TCGA-FY-A3TY	61	F	no	total	Rt	yes	4.2	0	6	T3	N0	MX	III	no	149.4768	alive	9	no
310	TCGA-FY-A3YR	64	F	no	total	Rt	yes	1.7	4	11	T3	N1a	MX	III	no	197.0061	alive	528	no
311	TCGA-FY-A40K	46	F	no	total	Rt	no	1	0	4	T1a	N0	MX	I	yes	151.8747	alive	461	no
312	TCGA-FY-A40L	55	F	no	total	Bilat	yes	1.7	2	3	T4a	N1b	MX	IV	no	220.3776	alive	200	no
313	TCGA-FY-A40M	51	F	no	total	Bilat	no	1.7	0	5	T1b	N0	MX	I	no	133.3997	alive	684	no
314	TCGA-FY-A4B0	76	M	no	total	Bilat	no	1	0	0	T1a	Nx	MX	I	no	226.7666	alive	1	no
315	TCGA-FY-A4B3	51	M	n.a	total	Bilat	yes	2.1	4	4	T3	N1a	MX	III	yes	208.4723	alive	41	no
316	TCGA-FY-A4B4	62	F	no	total	Rt	no	1.8	2	3	T1b	N1a	MX	III	no	125.7862	alive	657	no
317	TCGA-FY-A76V	54	M	no	total	Rt+Isthmus	no	3.1	0	0	T2	Nx	MX	II	no	173.5093	alive	51	no
318	TCGA-GE-A2C6	33	F	no	total	Bilat	yes	2.5	14	23	T3	N1b	MX	I	yes	246.3719	alive	381	no

319	TCGA-H2-A26U	54	F	no	total	Bilat	yes	1.5	0	7	T3	N0	MX	III	yes	201.9431	alive	517	no
320	TCGA-H2-A2K9	25	M	no	total	Bilat	no	2.8	11	14	T2	N1a	MX	I	no	211.7647	alive	659	no
321	TCGA-H2-A3RI	29	F	no	total	Lt	yes	2.7	0	5	T3	N0	MX	I	yes	201.328	alive	91	no
322	TCGA-H2-A421	34	F	no	total	Rt	no	1.5	2	3	T1b	N1a	M0	I	yes	142.8571	alive	610	no
323	TCGA-H2-A422	40	F	no	total	Rt	no	3	0	3	T2	N0	MX	I	no	256.2458	alive	566	no
324	TCGA-IM-A3EB	32	F	no	total	Rt	yes	3	3	12	T3	N1a	MX	I	yes	229.7658	alive	1488	no
325	TCGA-IM-A3ED	58	F	no	total	Lt	no	1.7	0	9	T1b	N0	MX	I	yes	280.5018	alive	1442	no
326	TCGA-IM-A420	39	F	no	total	Bilat	yes	4	5	14	T3	N1a	MX	I	no	174.8135	alive	90	no
327	TCGA-J8-A3NZ	52	F	no	total	Lt	yes	3.5	2	9	T3	N1a	M0	III	yes	225.0432	alive	524	no
328	TCGA-J8-A3O0	38	M	no	total	Rt	no	2.8	0	3	T2	N0	M0	I	no	235.9515	alive	1313	no
329	TCGA-J8-A3O1	33	F	no	total	Lt	no	6.5	12	19	T3	N1b	M0	I	no	516.9353	alive	827	no
330	TCGA-J8-A3O2	39	M	no	total	Lt	yes	3.5	23	106	T3	N1b	M0	I	no	241.272	alive	342	no
331	TCGA-J8-A3YD	47	F	n.a	total	Rt	no	1.5	2	6	T1b	N1a	M0	III	no	196.914	alive	968	no
332	TCGA-J8-A3YE	31	F	no	total	Lt	yes	3.5	1	4	T3	N1a	M0	I	yes	190.9875	alive	847	no
333	TCGA-J8-A3YF	83	M	no	total	Bilat	no	0.5	1	21	T1a	N1b	M0	IV	no	128.4355	alive	751	no
334	TCGA-J8-A3YG	54	F	no	total	Bilat	no	2.5	6	9	T2	N1a	M0	III	no	170.2406	alive	678	no
335	TCGA-J8-A3YH	39	M	no	total	Bilat	no	4.5	13	40	T3	N1b	M0	I	yes	137.6615	alive	175	no
336	TCGA-J8-A42S	45	M	n.a	total	Bilat	no	1.5	1	1	T1b	N1a	MX	III	no	203.2071	alive	75	no
337	TCGA-J8-A4HW	59	F	no	total	Lt	no	3	7	8	T2	N1a	M0	III	no	163.6364	alive	79	no
338	TCGA-J8-A4HY	68	F	no	total	Lt	yes	6.5	13	40	T4a	N1b	MX	IV	no	204.7993	alive	6	no
339	TCGA-KS-A41F	37	F	no	total	Lt	no	2.2	4	7	T2	N1a	M0	I	no	149.5902	alive	4367	no
340	TCGA-KS-A41J	28	F	no	total	Lt	no	2.3	2	2	T2	N1a	M0	I	yes	140.822	alive	477	no
341	TCGA-KS-A4I1	37	F	n.a	total	Bilat	no	2.2	0	4	T2	N0	M0	I	no	80.9411	alive	183	no

342	TCGA-KS-A4I3	41	M	no	total	Lt	yes	1.5	8	8	T3	N1a	M0	I	no	212.785	alive	600	no
343	TCGA-KS-A4I5	49	F	no	total	Rt	no	1.7	0	2	T1b	N0	M0	I	yes	134.1187	alive	205	no
344	TCGA-KS-A4I7	61	F	n.a	total	Lt	no	1.5	0	4	T1b	N0	M0	I	no	75.6915	alive	987	no
345	TCGA-KS-A4I9	46	F	n.a	total	Isthmus	no	1.7	0	5	T1b	N0	M0	I	yes	207.7518	alive	2658	no
346	TCGA-KS-A4IB	41	F	no	total	Lt	no	3.2	2	7	T2	N1a	M0	I	yes	206.1995	alive	2577	no
347	TCGA-KS-A4IC	45	F	no	total	Lt	no	1.1	2	4	T1b	N1a	M0	III	no	225.8031	alive	1073	no
348	TCGA-L6-A4EP	41	F	no	Lobectomy	Rt+Isthmus	no	3	0	3	T2	N0	M0	I	yes	83.7209	alive	1016	no
349	TCGA-L6-A4EQ	47	M	no	total	Lt	no	3.5	1	1	T2	N1a	M0	III	no	97.7836	alive	1228	no
350	TCGA-MK-A4N6	35	M	no	total	Rt	yes	8.2	40	77	T3	N1b	M0	I	yes	349.9425	alive	1098	no
351	TCGA-MK-A4N7	20	F	no	total	Isthmus	no	2.5	0	2	T2	N0	M0	I	yes	233.9181	alive	987	no
352	TCGA-MK-A4N9	41	F	no	total	Bilat	yes	2.6	1	9	T3	N1a	M0	I	yes	197.1922	alive	919	no
353	TCGA-QD-A8IV	50	F	no	total	Lt+Isthums	yes	1.6	15	38	T3	N1b	MX	IV	no	172.8538	alive	392	no

Figure 2. Overall survival of classic papillary thyroid carcinoma patients. (A) Age ≥ 45, (B) higher Tumor stage (T stage), and (C) higher American Joint Committee on Cancer (AJCC) stage were associated with poorer overall survival



BRAF mutation status and clinicopathological characteristics

Clinicopathological characteristics of classic PTC patients classified according to *BRAF* mutation status are shown in Table 2. None of the factors, including age, gender, thyroiditis, ETE, tumor size, AJCC stage, recurrence, or vital status, differed significantly between the $BRAF^{V600E}$ and wild-type *BRAF* patients.

Table 2. Clinicopathological characteristics of classic papillary thyroid carcinoma

patients with wild-type *BRAF and* $BRAF^{V600E}$

	Wild-type *BRAF* (n = 160)	$BRAF^{V600E}$ (n = 193)	p - value
Age, years			
< 45	78 (48.8%)	92 (49.7%)	0.924
≥ 45	82 (51.2%)	101 (52.3%)	
Gender			
Male	41 (25.6%)	55 (28.5%)	0.629
Female	119 (74.4%)	138 (71.5%)	
Thyroiditis			
No	47 (29.4%)	65 (33.7%)	1.000
Yes	49 (30.6%)	67 (34.7%)	
n.a	64 (40.0%)	61 (31.6%)	
Extrathyroidal extension			
No	115 (71.9%)	121 (62.7%)	0.087
Yes	45 (28.1%)	72 (37.3%)	
Tumor size, cm			
≤ 2.0	63 (39.4%)	83 (43.0%)	0.561

> 2.0	97 (60.6%)	110 (57.0%)	
T stage			
T1, T2	102 (63.8%)	110 (57.0%)	0.238
T3, T4	58 (36.2%)	83 (43.0%)	
N stage			
N0	44 (27.5%)	63 (32.6%)	0.572
N1	84 (52.5%)	93 (48.2%)	
Nx	32 (20.0%)	37 (19.2%)	
AJCC stage			
I, II	104 (65.0%)	129 (66.8%)	0.802
III, IV	56 (35.0%)	64 (33.2%)	
Recurrence			
No	149 (93.1%)	176 (91.2%)	0.637
Yes	11 (6.9%)	17 (8.8%)	
Vital Status			
Alive	155 (96.9%)	184 (95.3%)	0.643
Dead	5 (3.1%)	9 (4.7%)	

Abbreviations: AJCC = American Joint Committee on Cancer

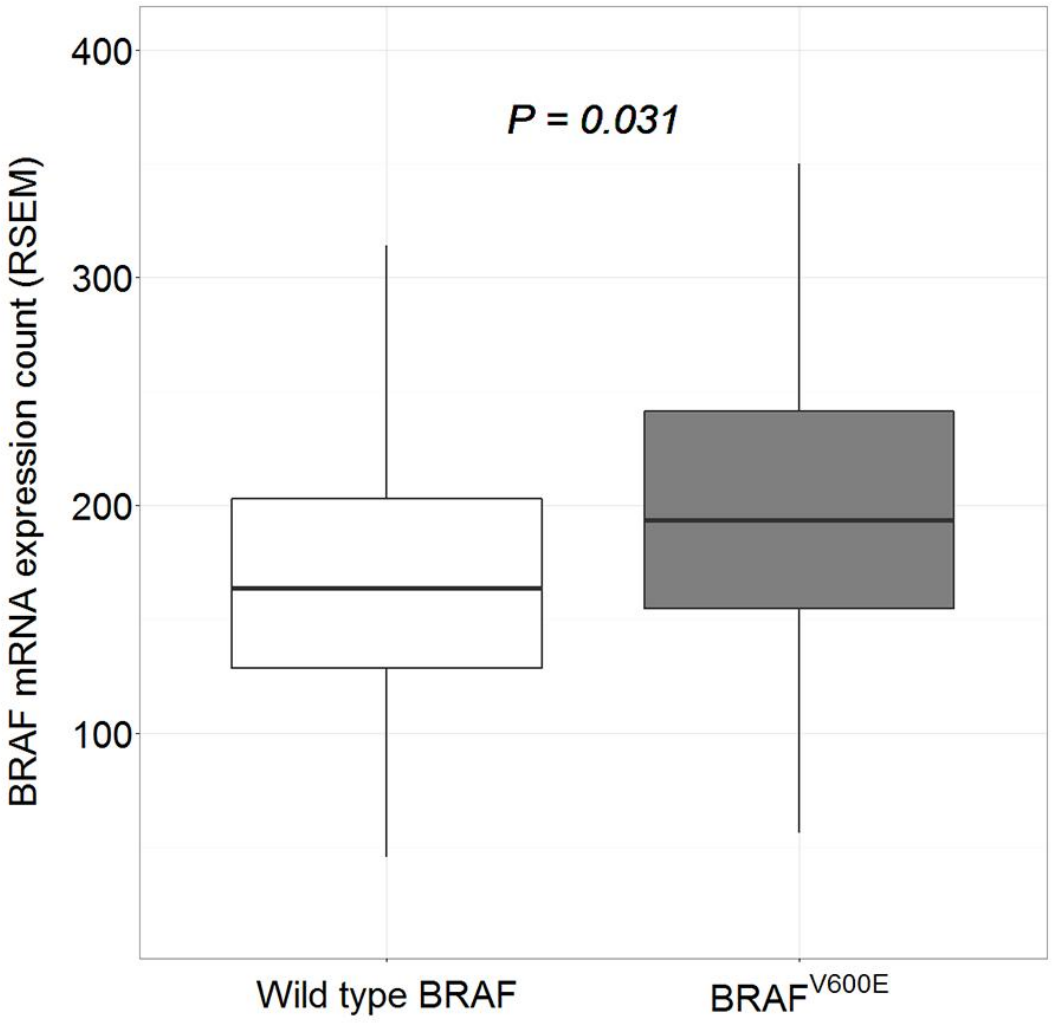
BRAF mRNA expression levels in wild-type *BRAF* and *BRAF*^{V600E}

classic PTC

The count data for the wild-type *BRAF* and $BRAF^{V600E}$ patients can be summarized as follows: for wild-type *BRAF* patients, minimum 45.3, lower quartile 128.7, median 166.2, mean 179.3 and upper quartile 204.2; for $BRAF^{V600E}$ patients, minimum 56.1, lower quartile 154.7, median 194.1, mean 197.6, and upper quartile 243.5 (Figure 3). The mean *BRAF* mRNA expression count was significantly higher in $BRAF^{V600E}$ patients than in patients with wild-type *BRAF* (197.6 vs. 179.3, p = 0.031).

Figure 3. *BRAF* mRNA expression counts in classic papillary thyroid carcinoma

patients. The mean *BRAF* mRNA expression count was significantly higher in $BRAF^{V600E}$ patients than in patients with wild-type *BRAF*



Clinical significance of the *BRAF* mRNA expression level in wild-

type *BRAF* and *BRAF*^{V600E} classic PTC

Table 3 shows the results of unpaired two sample t-test. *BRAF* mRNA expression was higher in patients < 45 years (199.0 vs. 180.2, p = 0.023), without thyroiditis (199.2 vs. 176.8, p = 0.032), with a tumor size > 2.0cm (196.2 vs. 179.4, p = 0.028), and with N1 nodal stage (197.6 vs. 176.0, p = 0.013). In wild-type *BRAF* patients, the *BRAF* mRNA expression count was higher in cases with a tumor size > 2 cm (189.4 vs. 163.8, p = 0.046). In addition, the count was higher in cases with N1 nodal stage (188.5 vs. 157.9, p = 0.040). Within *BRAF*^{V600E} patients, higher *BRAF* mRNA expression was associated with the presence of ETE (216.4 vs. 186.4, p = 0.001) and higher T stage (210.2 vs. 188.1, p = 0.016). The association between *BRAF* mRNA expression count and clinicopathological characteristics was summarized in Figure 4. Table 4 shows the results of linear regression analysis. The *BRAF* mRNA expression count was negatively correlated with age ≥ 45 years and thyroiditis (t-value -2.318, p = 0.021), and was positively correlated with tumor size > 2 cm (t-value 2.035, p = 0.043) and N1 nodal stage (t-value 2.314, p = 0.021). In wild-type *BRAF* patients, the *BRAF* mRNA expression count negatively correlated with age ≥ 45 years (t-value -2.001, p = 0.047) whereas it positively

correlated with presence of ETE (t-value 3.305, p = 0.001) and higher T stage (t-value 2.462, p = 0.015) in $BRAF^{V600E}$ patients.

Table 3. Unpaired t-test results of comparisons of each clinical variable, using *BRAF* mRNA expression counts as the response variable

	All (n=353)		Wild-type *BRAF* (n = 160)		*BRAF*^{V600E} (n = 193)	
	Mean (RSEM)	p-value	Mean (RSEM)	p-value	Mean (RSEM)	p-value
Age, years						
< 45	199.046	0.023	193.7	0.051	203.5	0.207
≥ 45	180.224		165.5		192.1	
Gender						
Male	183.224	0.310	168.3	0.271	194.4	0.647
Female	191.554		183.1		198.9	
Thyroiditis						
No	199.195	0.032	200.8	0.104	198.0	0.162
Yes	176.810		167.0		184.0	
ETE						
No	196.560	0.178	185.0	0.111	186.4	0.001
Yes	185.683		164.8		216.4	
Tumor size, cm						
≤ 2.0	179.442	0.028	163.8	0.046	191.4	0.209

> 2.0	196.233		189.4		202.3	
T stage						
T1, T2	183.554	0.079	178.7	0.908	188.1	0.016
T3, T4	197.910		180.3		210.2	
N stage						
N0	175.960	0.013	157.9	0.040	188.6	0.078
N1	197.587		188.5		205.8	
AJCC stage						
I, II	193.133	0.157	187.3	0.073	197.9	0.934
III, IV	181.824		164.4		197.0	
Recurrence						
No	187.747	0.191	177.7	0.364	196.3	0.424
Yes	207.181		201.0		211.2	
Vital Status						
Alive	188.792	0.574	179.4	0.639	196.7	0.563
Dead	201.301		174.2		216.4	

Abbreviations: RSEM = RNA-Seq by Expectation Maximization; ETE = extrathyroidal extension; AJCC = American Joint Committee on Cancer

Figure 4. *BRAF* mRNA expression counts according to clinicopathological features. In $BRAF^{V600E}$ patients, higher *BRAF* mRNA expression was associated with (A) the

presence of extrathyroidal extension and (B) higher Tumor stage (T stage). In wild-type *BRAF* patients, higher *BRAF* mRNA expression was associated with (C) tumor size > 2 cm and (D) N1 stage.

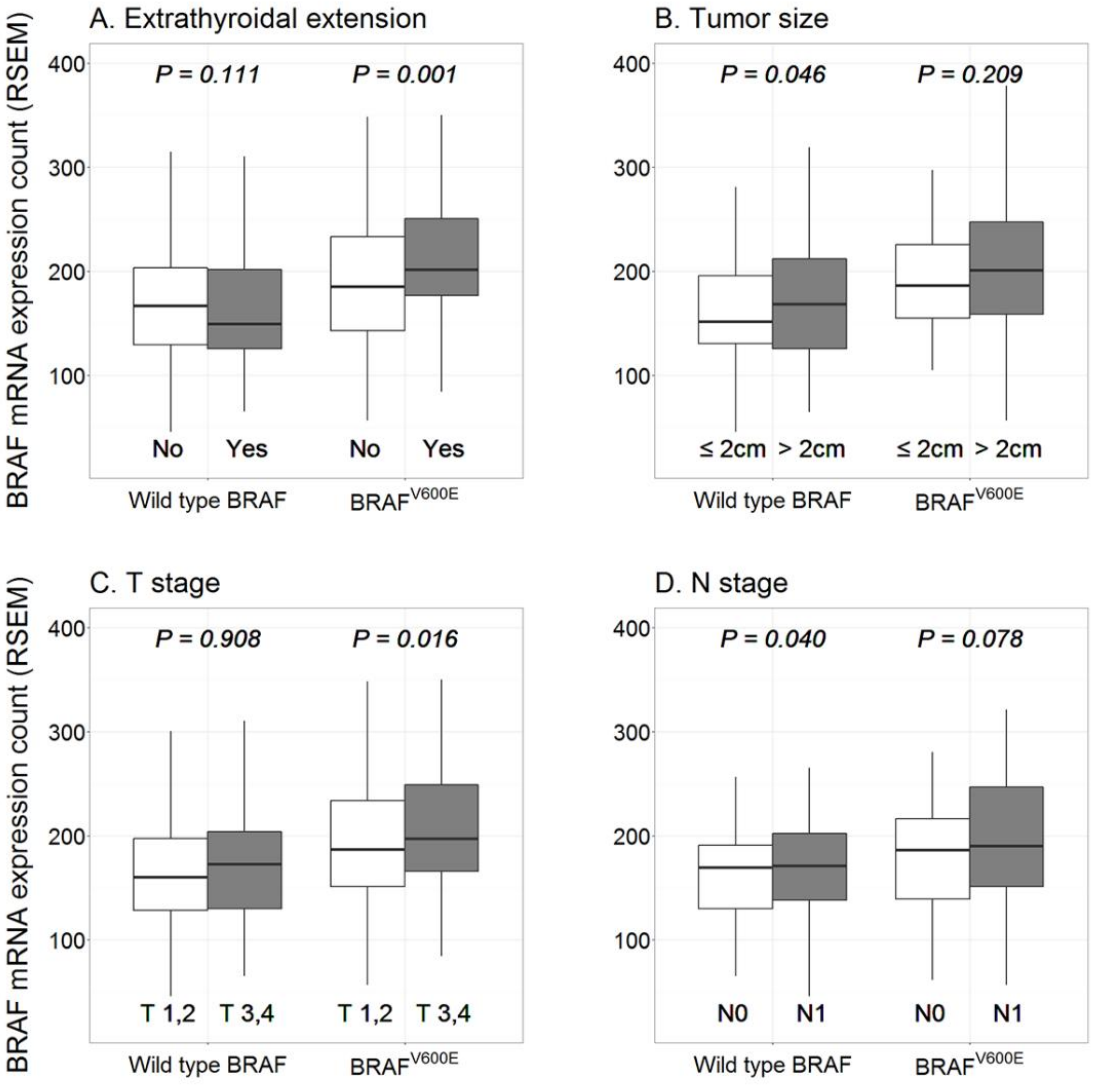


Table 4. Linear regression analysis of comparisons of each clinical variable, using *BRAF* mRNA expression counts as the response variable

	All (n=353)		Wild-type *BRAF* (n = 160)		*BRAF*^{V600E} (n = 193)	
	t-value	p-value	t-value	p-value	t-value	p-value
Age ≥ 45 years	-2.318	0.021	-2.001	0.047	-1.263	0.208
Male gender	0.908	0.365	0.909	0.365	0.448	0.655
Thyroiditis	-2.213	0.028	-1.855	0.066	-1.299	0.196
ETE	1.255	0.210	-1.281	0.202	3.305	0.001
Tumor size > 2.0 cm	2.035	0.043	1.772	0.078	1.201	0.231
T stage (T3, T4)	1.727	0.085	0.108	0.914	2.462	0.015
N stage (N1)	2.314	0.021	1.839	0.068	1.685	0.094
AJCC stage (III, IV)	-1.314	0.190	-1.538	0.126	-0.086	0.932
Recurrence	1.288	0.199	0.83	0.408	0.935	0.351
Mortality	0.598	0.551	-0.129	0.897	0.921	0.358

Abbreviations: ETE = extrathyroidal extension; AJCC = American Joint Committee on Cancer

Threshold level of the *BRAF* mRNA expression level associated with poor prognostic parameters

To calculate a threshold level of *BRAF*^{V600E} mRNA expression above which the expression levels correlate with the poor prognosis parameters available in the current dataset, Recursive Partitioning and Regression Trees were used. Threshold levels were only obtainable for the *BRAF* mutant group, not for the wild type-*BRAF* group. The results are shown in the Table 5. Cutoff RSEM value was 199.583. Of the 193 *BRAF*^{V600E} group patients, 103 had higher expression counts than cutoff value and 90 had lower expression counts than cutoff value. Odds ratio for extrathyroidal extension and size > 2cm was 3.655 and 1.755, respectively with marginal statistical significance and AUC of 0.627.

Table 5. Multivariate linear regression model based on *BRAF* mRNA cutoff value obtained from classification analysis. Backward selection method was applied to find fitted model. Threshold RSEM value of the PTC with *BRAF*^{V600E} was 199.583,

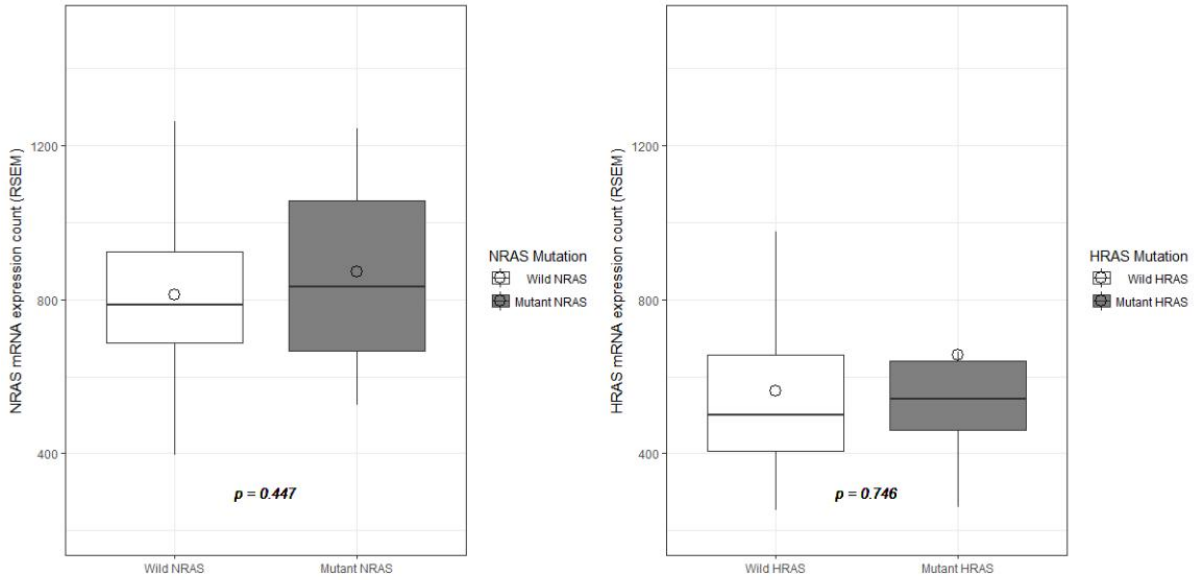
and AUC was 0.627

	Odds ratio	95% confidence interval	p-value
Extrathyroidal extension	3.655	0.952-17.976	0.074
Size (> 2cm)	1.755	0.968-3.209	0.065
T stage (T3, T4)	0.35	0.072-1.331	0.146

RAS mRNA expression levels according to RAS mutational status in PTC with wild-type BRAF

Of the PTCs with wild-type *BRAF*, *NRAS* and *HRAS* mutations were found in 15 and 6 patients, respectively. *KRAS* mutation was found in one patient. *RAS* mRNA expression levels according to *RAS* mutational status in PTC with wild-type BRAF were demonstrated in Figure 5. There was no significance difference in the *RAS* mRNA expression count between the wild-type and mutant *NRAS* PTC (821.9 vs. 871.8, p = 0.447). mRNA expression count of *HRAS* was also similar between the wild-type *HRAS* and mutant *HRAS* PTC (596.4 vs. 657.2, p = 0.746)

Figure 5. *RAS* mRNA expression counts according to *RAS* mutational status in PTC with wild-type BRAF. There was no significance difference in the *RAS* mRNA expression count between the wild-type and mutant *NRAS* PTC (821.9 vs. 871.8, p = 0.447).

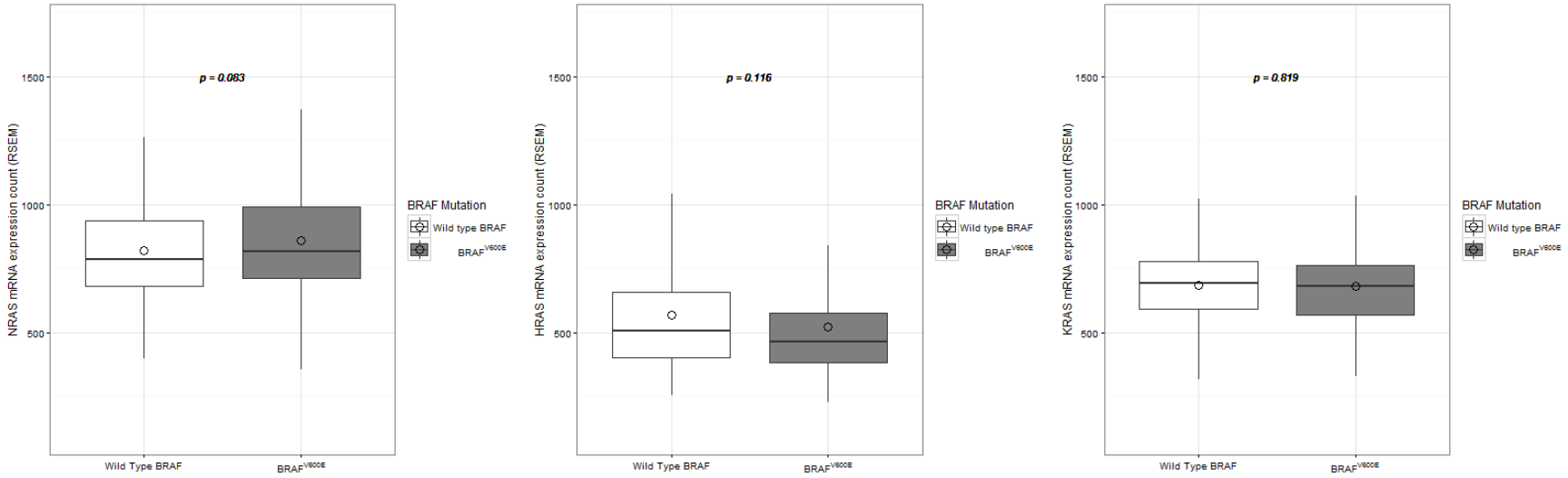


RAS mRNA expression levels in wild-type *BRAF* and *BRAF*^{V600E} PTC

RAS mRNA expression levels for the wild-type *BRAF* and *BRAF*^{V600E} patients was

shown in Figure 6. There was no difference in the *RAS* mRNA expression levels for the wild-type *BRAF* and $BRAF^{V600E}$ patients. The mean *NRAS* mRNA expression count in was 819.7 and 862.0 in wild-type and $BRAF^{V600E}$ PTC, respectively (p = 0.083). The mean *HRAS* mRNA expression count in was 591.7 and 540.1 in wild-type and $BRAF^{V600E}$ PTC, respectively (p = 0.116). The mean *KRAS* mRNA expression count in was 686.0 and 681.8 in wild-type and $BRAF^{V600E}$ PTC, respectively (p = 0.819).

Figure 6. *RAS* mRNA expression counts according to BRAF mutational status



Clinical significance of the *RAS* mRNA expression level in wild-type *BRAF* PTC

Table 6 shows the results of unpaired two sample t-test comparing clinicopathological features and *RAS* mRNA expression count in PTC with wild-type *BRAF*. Higher *NRAS* mRNA expression count was higher in patients < 45 years than in patients ≥ 45 years (864.6 vs. 777.1, p = 0.014). *NRAS* mRNA expression count was not associated with the other clinicopathological features including gender, thyroiditis, ETE, tumor size, T stage, N stage, AJCC stage, recurrence, or vital status. *HRAS* mRNA expression count was higher in the alive patients than in the dead patients (597.1 vs. 423.4, p = 0.003). For the mRNA expression count of *KRAS*, there was no correlation with clinicopathological features.

Table 6. Unpaired t-test results of comparisons of each clinical variable, using *RAS*

mRNA expression counts as the response variable in PTC with wild-type BRAF

	NRAS		*HRAS*		*KRAS*	
	Mean (RSEM)	p-value	Mean (RSEM)	p-value	Mean (RSEM)	p-value
Age, years						
< 45	864.562	0.014	597.547	0.822	701.822	0.233
≥ 45	777.093		586.14		670.903	
Gender						
Male	769.629	0.117	595.738	0.912	665.395	0.368
Female	836.997		590.31		693.067	
Thyroiditis						
No	798.572	0.156	601.07	0.391	664.694	0.084
Yes	863.069		552.356		722.154	
ETE						
No	823.973	0.738	575.056	0.259	693.089	0.432
Yes	808.903		634.239		667.799	
Tumor size, cm						
≤ 2.0	782.526	0.062	553.805	0.176	677.301	0.569

> 2.0	843.9		616.314		691.611	
T stage						
T1, T2	821.951	0.879	581.84	0.582	684.965	0.922
T3, T4	815.836		609.044		687.754	
N stage						
N0	776.629	0.074	576.219	0.66	665.37	0.518
N1	846.347		600.847		684.536	
AJCC stage						
I, II	840.213	0.121	588.634	0.858	698.991	0.198
III, IV	781.701		597.398		661.806	
Recurrence						
No	824.739	0.232	597.29	0.289	688.423	0.526
Yes	751.944		515.996		652.839	
Vital Status						
Alive	810.004	0.1	597.131	0.003	681.07	0.057
Dead	1121.361		423.374		838.072	

Abbreviations: RSEM = RNA-Seq by Expectation Maximization; ETE = extrathyroidal extension; AJCC = American Joint Committee on Cancer

Clinical significance of the *RAS* mRNA expression level in *BRAF*^{V600E} PTC

Table 7 shows the results of unpaired two sample t-test comparing clinicopathological features and *RAS* mRNA expression count in PTC with $BRAF^{V600E}$. Higher *NRAS* mRNA expression count was associated with presence of thyroiditis (905.0 vs. 785.6, p = 0.001), ETE (921.6 vs. 826.5, p = 0.007), higher T stage (905.2 vs. 829.3, p = 0.024), and N1 nodal stage (906.0 vs. 822.0, p = 0.017). The mRNA expression count of *HRAS* was higher in the patients without ETE (580.9 vs. 471.5, p = 0.004) and lower T stage (581.4 vs. 485.3, p = 0.015). The mRNA expression count of *KRAS* was higher in the patients with ETE (738.8 vs. 647.9, p = 0.001), higher T stage (724.2 vs. 649.8, p = 0.005), N1 stage (717.5 vs. 653.3, p = 0.018), and higher AJCC stage (721.9 vs. 661.9, p = 0.042).

Table 7 Unpaired t-test results of comparisons of each clinical variable, using *RAS* mRNA expression counts as the response variable in PTC with $BRAF^{V600E}$

	NRAS		*HRAS*		*KRAS*	
	Mean	p-value	Mean	p-value	Mean	p-value

	(RSEM)		(RSEM)		(RSEM)	
Age, years						
< 45	880.236	0.289	521.589	0.403	670.821	0.407
≥ 45	845.307		556.906		691.845	
Gender						
Male	897.885	0.223	526.194	0.657	689.849	0.701
Female	847.638		545.602		678.625	
Thyroiditis						
No	785.563	0.001	523.829	0.477	654.187	0.178
Yes	905.04		560.287		688.14	
ETE						
No	826.491	0.007	580.89	0.004	647.903	0.001
Yes	921.559		471.472		738.828	
Tumor size, cm						
≤ 2.0	857.383	0.801	496.587	0.052	688.124	0.65
> 2.0	865.408		572.881		677.07	
T stage						
T1, T2	829.324	0.024	581.425	0.015	649.827	0.005

T3, T4	905.206		485.264		724.229	
N stage						
N0	821.957	0.017	569.787	0.236	653.265	0.018
N1	906		506.271		717.516	
AJCC stage						
I, II	850.384	0.333	545.471	0.692	661.935	0.042
III, IV	885.284		529.186		721.911	
Recurrence						
No	856.054	0.346	538.218	0.769	678.244	0.45
Yes	923.069		559.251		718.886	
Vital Status						
Alive	860.667	0.72	541.51	0.671	676.485	0.336
Dead	888.333		510.654		790.961	

Abbreviations: RSEM = RNA-Seq by Expectation Maximization; ETE = extrathyroidal extension; AJCC = American Joint Committee on Cancer

Discussion

Although TCGA genomic data are archived under standardized and strictly controlled condition, some concerns remain regarding the correlation of genomic

data with clinical information due to the potential inaccuracy of the clinical information. Such potential discrepancies exist because the surgical procedures and pathologic reporting systems differed among the institutes that collected the data. Therefore, to ensure the reliability of the TCGA data, each scanned original pathologic file was examined and revised if there was missing information. Moreover, to examine the reliability of the TCGA data, survival analysis was perfomed; the results showed that overall survival was associated with older patient age, absence of thyroiditis, higher T stage, and higher AJCC stage, consistent with the known relationships between these parameters and overall survival in PTC.

The MAPK pathway [Ras → Raf → MEK → MAPK/ERK] is a fundamental intracellular signaling pathway that plays a central role in cellular functions such as proliferation, differentiation, apoptosis, and survival [29]. Several mutations in the MAPK pathway are involved in the tumorigenesis of PTC. Of these, the *BRAF*^{V600E} and *RAS* mutations are the most common activators of the MAPK pathway [30]. The *BRAF*^{V600E} kinase is more active than the wild-type *BRAF*, and its transformation efficacy is 138- to 500-fold higher [31, 32]. The clinical impact of the *BRAF*^{V600E} mutation is well known, but the significance of the *BRAF* mRNA

expression level has not been previously studied well.

Although there are concerns that the mRNA expression level may not provide a true reflection of the protein expression level, RNA sequencing has the advantage in that the results obtained using this method can be objectively measured and there are studies reporting positive correlation between the mRNA expression level and protein expression level. One study showed that differentially expressed mRNA correlated well with their protein levels [33] and another study showed that more than 85% of the variation in steady-state protein levels could be explained by changes in mRNA levels [34]. In this regard, the *BRAF* and *RAS* mRNA expression level was used as surrogate markers for Braf and Ras function in thyroid carcinoma, although a substantial proportion of variation in the protein level cannot be explained wholly by changes in the mRNA level alone [35].

The significance of *BRAF* mRNA expression in thyroid tumors has been reported previously [36], and recurrence or distant metastasis in PTC patients has been shown to be related to higher *BRAF* mRNA expression [36]. In the present study, there was an association between the *BRAF* mRNA expression level and clinicopathological characteristics in a large number of cases. It was found that higher *BRAF* mRNA expression levels were associated with aggressive clinical

features in both wild-type *BRAF* and $BRAF^{V600E}$ patients except patient age < 45 years which is controversial as a cutoff for favorable prognosis [37, 38]. On the other hand, no differences were observed in the clinicopathological characteristics of wild-type *BRAF* and $BRAF^{V600E}$ patients, which is in contrast to the results of other studies showing that the $BRAF^{V600E}$ mutation is associated with poor prognostic factors [39]. This might be because a relatively small number of patients with short term follow-up weakened the statistical power. It can be also postulated that the expression level of *BRAF* mRNA contributes to aggressiveness of PTC, and PTC with $BRAF^{V600E}$ may not exhibit aggressive features unless mRNA expression of the $BRAF^{V600E}$ reaches a certain level. Additionally, the present dataset might have included relatively low numbers of PTCs with high $BRAF^{V600E}$ mRNA expression counts, which would have prevented observing a statistical difference in clinicopathological features between wild-type *BRAF* and $BRAF^{V600E}$ patients. In fact, a number of studies with small sample size could not demonstrate that *BRAF* mutation is a poor prognostic factor [12, 13, 40, 41], whereas studies with larger sample sizes could [10, 42].

The significance of the Braf protein expression level has been demonstrated in previous studies using immunohistochemistry. Immunohistochemical

studies suggest that higher Braf protein expression is associated with poor prognostic factors in PTC and melanoma. Zagzag et al. [23] performed an immunohistochemical study using a mutation-specific antibody (VE1; Springer-Bio, Maisons-Alfort Cedex, France) and reported that higher $Braf^{V600E}$ mutant protein expression is associated with the presence of ETE in PTC. Although immunohistochemical studies are difficult to evaluate objectively or quantitatively, and the results of such studies are sometimes not reproducible [43], detection of $Braf^{V600E}$ mutant protein using a mutation-specific antibody has been validated in several studies [44-46]. An association between high Braf expression and adverse prognostic outcomes was also demonstrated in melanoma [47].

The possible heterogeneous distribution of the *BRAF* mutation in PTC tumors is an important consideration in interpreting *BRAF* mRNA expression. Whether the *BRAF* mutation in PTC is clonal or subclonal has been the subject of much debate. Up until recently, the general perception was that *BRAF* mutations in PTC are strong driver mutations and PTCs with the *BRAF* mutation are clonal. This viewpoint is supported by the results of an immunohistochemical study using a $Braf^{V600E}$ mutation-specific antibody [48] as well by a recent publication from the TCGA Research Network [27]. Conversely, a number of recent studies suggest that

tumors are heterogeneous for the *BRAF* mutation and that not every cell in a *BRAF* mutation-positive tumor has a heterozygous mutation [24, 25, 49-51]. These studies also demonstrated that the percentage of *BRAF* mutant alleles in PTC with the $BRAF^{V600E}$ mutation were significantly below 50%, the theoretical percentage for a clonal heterozygous mutation [24, 49-51]. Additionally, Guerra et al. subsequently demonstrated that a higher percentage of *BRAF* mutant alleles are associated with older age, larger tumor sizes, and higher recurrence rates in PTC [25]. Other studies demonstrated that a higher proportion of *BRAF* mutant cells in PTC is associated with larger tumor size [51, 52] and lymph node metastasis [51]. Likewise, the present study showed that high *BRAF* mRNA expression correlates with aggressive clinical features of PTC, probably because the tumors with high *BRAF* mRNA expression had a greater number of $BRAF^{V600E}$ mutant cells. Further molecular investigations, including single cell sequencing, might help resolve the heterogeneity issue. If the *BRAF* mutation proves to be heterogeneous in PTC, it may turn out that PTCs cannot be categorized merely on whether they are *BRAF* mutated or not.

Regarding the role of *BRAF* mRNA expression in wild-type PTC, the issue of tumor heterogeneity may have little impact on the aggressive clinicopathological

characteristics. There may be increased activities of the Braf kinase in association with presumptive increased Braf protein levels as a result of the overexpression of wild-type *BRAF* mRNA in aggressive PTC compared with indolent PTC. Consequently, the downstream signaling activities of the MAP kinase pathway may be more active in aggressive PTC than in indolent PTC; this would be consistent with the fact that overexpression of wild-type *BRAF* also causes increased activation of downstream signaling of the MAP kinase pathway [53-55]. This may explain the finding in the present study that *BRAF* mRNA overexpression was associated with aggressive features even in PTC with the wild-type *BRAF*. The clinical significance of this finding needs further studies to define. It is also interesting that in this study the mean *BRAF* mRNA expression level was significantly higher in PTC with $BRAF^{V600E}$ than in PTC with the wild-type *BRAF* although there was no significant difference in the tumor aggressiveness between the two groups. The roles of the wild-type *BRAF* and $BRAF^{V600E}$ in tumor progression are apparently complex. Further mechanistic studies are needed to fully understand these findings.

Meanwhile, various *BRAF* inhibitors are currently being tested in clinical trials on patients with refractory thyroid carcinoma, with varying results.

Subclonality, splice variants, or copy number gains of *BRAF* may be responsible for the lack of responses to *BRAF* inhibitors in many cases [56-58]. In selecting patients who may benefit from *BRAF* inhibitors, the *BRAF* mRNA expression level might serve as a potentially useful selection indicator if, in the future, it is proven to be specifically correlated with the response rate to *BRAF* inhibitors. As such, *BRAF* mRNA expression level might be helpful in predicting therapeutic effects. Similarly, it could also help exclude patients who would likely not respond to unnecessary treatments with *BRAF* inhibitors, and thereby avoid unnecessary exposure of such patients to the undesirable side effects of these drugs.

It is well-known that that the *RAS* mutation-positive PTC is less aggressive because they are usually follicular variant PTC which is less likely to have lymph node metastasis or ETE [59]. However, the role of *RAS* mutations as prognostic molecular markers for classical PTC is not established. In this study, the mRNA expression count of *RAS* was similar regardless of mutational status of BRAF or *RAS*. On the other hand, the mRNA expression count of *NRAS* and *KRAS* was associated with poor prognostic factors such as ETE, higher T stage, and N1 stage in the PTC with $BRAF^{V600E}$. In contrast, the mRNA expression count of *HRAS* was associated with the absence of ETE and lower T stage. The significance of the

RAS mRNA expression level in PTC should be studied in the future.

In conclusion, high *BRAF* mRNA expression levels were associated with aggressive clinical features in both wild-type *BRAF* and *BRAF* ^{V600E} patients with classic PTC, although the association with recurrence or survival could not be shown due to limited follow-up. Although heterogeneity of the *BRAF* mutation could weaken the correlation between the *BRAF* mRNA expression level and aggressive clinical features, the correlation was probably more robust in the PTC samples with higher percentages of *BRAF* mutant cells. Evaluation of the *BRAF* and *RAS* mRNA expression level in patients could provide a way to establish more accurate prognoses and risk stratification.

References

1. Siegel R, Ma J, Zou Z, Jemal A Cancer statistics, 2014. CA Cancer J Clin 2014;64:9-29.
2. Hundahl SA, Fleming ID, Fremgen AM, Menck HR A National Cancer Data Base report on 53,856 cases of thyroid carcinoma treated in the U.S., 1985-1995 [see commetns]. Cancer 1998;83:2638-48.

3. Davies L, Welch HG Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA 2006;295:2164-7.

4. Xing M BRAF mutation in thyroid cancer. Endocr Relat Cancer 2005;12:245-62.

5. Xing M, Westra WH, Tufano RP, Cohen Y, Rosenbaum E, Rhoden KJ, et al. BRAF mutation predicts a poorer clinical prognosis for papillary thyroid cancer. J Clin Endocrinol Metab 2005;90:6373-9.

6. Xing M, Alzahrani AS, Carson KA, Shong YK, Kim TY, Viola D, et al. Association Between BRAF V600E Mutation and Recurrence of Papillary Thyroid Cancer. J Clin Oncol 2015;33:42-50.

7. Abubaker J, Jehan Z, Bavi P, Sultana M, Al-Harbi S, Ibrahim M, et al. Clinicopathological analysis of papillary thyroid cancer with PIK3CA alterations in a Middle Eastern population. J Clin Endocrinol Metab 2008;93:611-8.

8. Lang BH, Chai YJ, Cowling BJ, Min HS, Lee KE, Youn YK Is BRAFV600E mutation a marker for central nodal metastasis in small papillary thyroid carcinoma? Endocr Relat Cancer 2014;21:285-95.

9. Xing M BRAF mutation in papillary thyroid cancer: pathogenic role,

molecular bases, and clinical implications. Endocr Rev 2007;28:742-62.

10. Kim TH, Park YJ, Lim JA, Ahn HY, Lee EK, Lee YJ, et al. The association of the BRAF(V600E) mutation with prognostic factors and poor clinical outcome in papillary thyroid cancer: a meta-analysis. Cancer 2012;118:1764-73.

11. Xing M Prognostic utility of BRAF mutation in papillary thyroid cancer. Mol Cell Endocrinol 2010;321:86-93.

12. Walczyk A, Kowalska A, Kowalik A, Sygut J, Wypiorkiewicz E, Chodurska R, et al. The BRAF(V600E) mutation in papillary thyroid microcarcinoma: does the mutation have an impact on clinical outcome? Clin Endocrinol (Oxf) 2014;80:899-904.

13. Ito Y, Yoshida H, Maruo R, Morita S, Takano T, Hirokawa M, et al. BRAF mutation in papillary thyroid carcinoma in a Japanese population: its lack of correlation with high-risk clinicopathological features and disease-free survival of patients. Endocr J 2009;56:89-97.

14. Davis RJ The mitogen-activated protein kinase signal transduction pathway. J Biol Chem 1993;268:14553-6.

15. Howell GM, Hodak SP, Yip L RAS mutations in thyroid cancer. Oncologist

2013;18:926-32.

16. Nikiforov YE, Nikiforova MN Molecular genetics and diagnosis of thyroid cancer. Nat Rev Endocrinol 2011;7:569-80.

17. Bos JL ras oncogenes in human cancer: a review. Cancer Res 1989;49:4682-9.

18. Garcia-Rostan G, Zhao H, Camp RL, Pollan M, Herrero A, Pardo J, et al. ras mutations are associated with aggressive tumor phenotypes and poor prognosis in thyroid cancer. J Clin Oncol 2003;21:3226-35.

19. Fukahori M, Yoshida A, Hayashi H, Yoshihara M, Matsukuma S, Sakuma Y, et al. The associations between RAS mutations and clinical characteristics in follicular thyroid tumors: new insights from a single center and a large patient cohort. Thyroid 2012;22:683-9.

20. Song YS, Lim JA, Park YJ Mutation Profile of Well-Differentiated Thyroid Cancer in Asians. Endocrinol Metab (Seoul) 2015;30:252-62.

21. Giordano TJ, Kuick R, Thomas DG, Misek DE, Vinco M, Sanders D, et al. Molecular classification of papillary thyroid carcinoma: distinct BRAF, RAS, and RET/PTC mutation-specific gene expression profiles discovered by DNA microarray analysis. Oncogene 2005;24:6646-56.

22. Xing M Molecular pathogenesis and mechanisms of thyroid cancer. Nat Rev Cancer 2013;13:184-99.

23. Zagzag J, Pollack A, Dultz L, Dhar S, Ogilvie JB, Heller KS, et al. Clinical utility of immunohistochemistry for the detection of the BRAF v600e mutation in papillary thyroid carcinoma. Surgery 2013;154:1199-204; discussion 204-5.

24. Kim MH, Bae JS, Lim DJ, Lee H, Jeon SR, Park GS, et al. Quantification of BRAF V600E alleles predicts papillary thyroid cancer progression. Endocr Relat Cancer 2014;21:891-902.

25. Guerra A, Fugazzola L, Marotta V, Cirillo M, Rossi S, Cirello V, et al. A high percentage of BRAFV600E alleles in papillary thyroid carcinoma predicts a poorer outcome. J Clin Endocrinol Metab 2012;97:2333-40.

26. Chai YJ, Kim YA, Jee HG, Yi JW, Jang BG, Lee KE, et al. Expression of the embryonic morphogen Nodal in differentiated thyroid carcinomas: Immunohistochemistry assay in tissue microarray and The Cancer Genome Atlas data analysis. Surgery 2014;156:1559-68.

27. Integrated genomic characterization of papillary thyroid carcinoma. Cell 2014;159:676-90.

28. Li B, Dewey CN RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. BMC Bioinformatics 2011;12:323.

29. Steelman LS, Abrams SL, Shelton JG, Chappell WH, Basecke J, Stivala F, et al. Dominant roles of the Raf/MEK/ERK pathway in cell cycle progression, prevention of apoptosis and sensitivity to chemotherapeutic drugs. Cell Cycle 2010;9:1629-38.

30. Kimura ET, Nikiforova MN, Zhu Z, Knauf JA, Nikiforov YE, Fagin JA High prevalence of BRAF mutations in thyroid cancer: genetic evidence for constitutive activation of the RET/PTC-RAS-BRAF signaling pathway in papillary thyroid carcinoma. Cancer Res 2003;63:1454-7.

31. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature 2002;417:949-54.

32. Wan PT, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM, et al. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;116:855-67.

33. Koussounadis A, Langdon SP, Um IH, Harrison DJ, Smith VA Relationship between differentially expressed mRNA and mRNA-protein correlations in a xenograft model system. Sci Rep 2015;5:10775.

34. Csardi G, Franks A, Choi DS, Airoldi EM, Drummond DA Accounting for experimental noise reveals that mRNA levels, amplified by post-transcriptional processes, largely determine steady-state protein levels in yeast. PLoS Genet 2015;11:e1005206.

35. Vogel C, Marcotte EM Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet 2012;13:227-32.

36. Araujo PP, Marcello MA, Tincani AJ, Guilhen AC, Morari EC, Ward LS mRNA BRAF expression helps to identify papillary thyroid carcinomas in thyroid nodules independently of the presence of BRAFV600E mutation. Pathol Res Pract 2012;208:489-92.

37. Nixon IJ, Wang LY, Migliacci JC, Eskander A, Campbell MJ, Aniss A, et al. An International Multi-Institutional Validation of Age 55 Years as a Cutoff for Risk Stratification in the AJCC/UICC Staging System for Well-Differentiated Thyroid Cancer. Thyroid 2016;26:373-80.

38. Kim SJ, Myong JP, Suh H, Lee KE, Youn YK Optimal Cutoff Age for Predicting Mortality Associated with Differentiated Thyroid Cancer. PLoS One 2015;10:e0130848.

39. Xing M, Alzahrani AS, Carson KA, Viola D, Elisei R, Bendlova B, et al.

Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. JAMA 2013;309:1493-501.

40. Nam JK, Jung CK, Song BJ, Lim DJ, Chae BJ, Lee NS, et al. Is the BRAF(V600E) mutation useful as a predictor of preoperative risk in papillary thyroid cancer? Am J Surg 2012;203:436-41.

41. Ahn D, Park JS, Sohn JH, Kim JH, Park SK, Seo AN, et al. BRAFV600E mutation does not serve as a prognostic factor in Korean patients with papillary thyroid carcinoma. Auris Nasus Larynx 2012;39:198-203.

42. Li F, Chen G, Sheng C, Gusdon AM, Huang Y, Lv Z, et al. BRAFV600E mutation in papillary thyroid microcarcinoma: a meta-analysis. Endocr Relat Cancer 2015;22:159-68.

43. Koperek O, Kornauth C, Capper D, Berghoff AS, Asari R, Niederle B, et al. Immunohistochemical detection of the BRAF V600E-mutated protein in papillary thyroid carcinoma. Am J Surg Pathol 2012;36:844-50.

44. Zimmermann AK, Camenisch U, Rechsteiner MP, Bode-Lesniewska B, Rossle M Value of immunohistochemistry in the detection of BRAF(V600E) mutations in fine-needle aspiration biopsies of papillary thyroid carcinoma. Cancer Cytopathol 2014;122:48-58.

45. Crescenzi A, Guidobaldi L, Nasrollah N, Taccogna S, Cicciarella Modica DD, Turrini L, et al. Immunohistochemistry for BRAF(V600E) antibody VE1 performed in core needle biopsy samples identifies mutated papillary thyroid cancers. Horm Metab Res 2014;46:370-4.

46. Ilie MI, Lassalle S, Long-Mira E, Bonnetaud C, Bordone O, Lespinet V, et al. Diagnostic value of immunohistochemistry for the detection of the BRAF(V600E) mutation in papillary thyroid carcinoma: comparative analysis with three DNA-based assays. Thyroid 2014;24:858-66.

47. Safaee Ardekani G, Jafarnejad SM, Khosravi S, Martinka M, Ho V, Li G Disease progression and patient survival are significantly influenced by BRAF protein expression in primary melanoma. Br J Dermatol 2013;169:320-8.

48. Ghossein RA, Katabi N, Fagin JA Immunohistochemical detection of mutated BRAF V600E supports the clonal origin of BRAF-induced thyroid cancers along the spectrum of disease progression. J Clin Endocrinol Metab 2013;98:E1414-21.

49. Guerra A, Sapio MR, Marotta V, Campanile E, Rossi S, Forno I, et al. The primary occurrence of BRAF(V600E) is a rare clonal event in papillary

thyroid carcinoma. J Clin Endocrinol Metab 2012;97:517-24.

50. Cheng SP, Hsu YC, Liu CL, Liu TP, Chien MN, Wang TY, et al. Significance of allelic percentage of BRAF c.1799T > A (V600E) mutation in papillary thyroid carcinoma. Ann Surg Oncol 2014;21 Suppl 4:S619-26.

51. Finkel A, Liba L, Simon E, Bick T, Prinz E, Sabo E, et al. Subclonality for BRAF Mutation in Papillary Thyroid Carcinoma Is Associated With Earlier Disease Stage. J Clin Endocrinol Metab 2016;101:1407-13.

52. de Biase D, Cesari V, Visani M, Casadei GP, Cremonini N, Gandolfi G, et al. High-sensitivity BRAF mutation analysis: BRAF V600E is acquired early during tumor development but is heterogeneously distributed in a subset of papillary thyroid carcinomas. J Clin Endocrinol Metab 2014;99:E1530-8.

53. Kondo T, Nakazawa T, Murata S, Kurebayashi J, Ezzat S, Asa SL, et al. Enhanced B-Raf protein expression is independent of V600E mutant status in thyroid carcinomas. Hum Pathol 2007;38:1810-8.

54. Mitsutake N, Miyagishi M, Mitsutake S, Akeno N, Mesa C, Jr., Knauf JA, et al. BRAF mediates RET/PTC-induced mitogen-activated protein kinase activation in thyroid cells: functional support for requirement of the RET/PTC-RAS-BRAF pathway in papillary thyroid carcinogenesis.

Endocrinology 2006;147:1014-9.

55. Ouyang B, Knauf JA, Smith EP, Zhang L, Ramsey T, Yusuff N, et al. Inhibitors of Raf kinase activity block growth of thyroid cancer cells with RET/PTC or BRAF mutations in vitro and in vivo. Clin Cancer Res 2006;12:1785-93.

56. Xing M BRAFV600E mutation and papillary thyroid cancer: chicken or egg? J Clin Endocrinol Metab 2012;97:2295-8.

57. Poulikakos PI, Persaud Y, Janakiraman M, Kong X, Ng C, Moriceau G, et al. RAF inhibitor resistance is mediated by dimerization of aberrantly spliced BRAF(V600E). Nature 2011;480:387-90.

58. Shi H, Moriceau G, Kong X, Lee MK, Lee H, Koya RC, et al. Melanoma whole-exome sequencing identifies (V600E)B-RAF amplification-mediated acquired B-RAF inhibitor resistance. Nat Commun 2012;3:724.

59. Medici M, Kwong N, Angell TE, Marqusee E, Kim MI, Frates MC, et al. The variable phenotype and low-risk nature of RAS-positive thyroid nodules. BMC Med 2015;13:184.

국문초록

갑상선유두암에서의 *BRAF* 와 *RAS* 유전자 mRNA 발현양의 임상적 의의: The Cancer Genome Atlas 분석

서울대학교 대학원

의학과 외과학 전공

채 영 준

<배경 및 목적> *BRAF* 유전자와 *RAS* 유전자 돌연변이는 갑상선유두암에서 가장 흔한 두 가지 유전자 돌연변이이며, *BRAF* 유전자 돌연변이가 존재하는 갑상선유두암은 나쁜 예후를 갖는다고 알려져 있다. 그러나 각 유전자의 mRNA 발현양과 갑상선유두암의 예후 또는 예후인자와의 연관성에 대해서는 알려진 바가 없다. 이에 본 연구에서는 The Cancer Genome Atlas (TCGA) database 에 등록되어 있는 갑상선유두암 환자들의 *BRAF* 유전자와 *RAS* 유전자의 mRNA 발현양을 분석하고 그 임상적 의미에 대해 분석하고자 한다.

<대상 및 방법 TCGA database에서 499명의 갑상선암환자 가운데 변이형갑상선유두

암을 제외하고 353명의 전형적갑상선유두암 (classic papillary thyroid carcinoma) 환자의 데이터를 다운로드 하였다. mRNA level 은 RNA-Seq with the Expectation Maximization algorithm 을 이용하여 분석하였다.

<결과> BRAF 돌연변이 여부는 전형적갑상선유두암의 예후인자와 연관이 없었다. *BRAF* mRNA level은 $BRAF^{V600E}$ 돌연변이가 있는 군이 그렇지 않은 군에 비하여 유의하게 높았다 (197.6 vs. 179.3, p = 0.031). 야생형 *BRAF* 환자에서 *BRAF* mRNA level은 암의 크기가 2cm를 넘는 군에서 그렇지 않은 군에 비에서 높았으며 (189.4 vs. 163.8, p = 0.046), 림프절 전이가 있는 군에서 그렇지 않은 군보다 높았다 (188.5 vs. 157.9, p = 0.040). $BRAF^{V600E}$ 돌연변이 군에서는 *BRAF* mRNA level은 피막외침범 (216.4 vs 186.4, p = 0.001) 및 높은 T-병기와 연관이 있었다 (210.2 vs. 188.1, p = 0.016). 또한 $BRAF^{V600E}$ 돌연변이 군에서, *NRAS* mRNA level은 피막외침범 (921.6 vs. 826.5, p = 0.007), 높은 T-병기 (905.2 vs. 829.3, p = 0.024), 림프절 전이와 연관이 있었다 (906.0 vs. 822.0, p = 0.017). *KRAS* mRNA level은 피막외침범 (738.8 vs. 647.9, p = 0.001), 높은 T-병기 (724.2 vs. 649.8, p = 0.005), 림프절 전이 (717.5 vs. 653.3, p = 0.018), 높은 AJCC 병기 (721.9 vs. 661.9, p = 0.042)와 연관이 있었다.

<결론> *BRAF* mRNA 발현양은 *BRAF* 돌연변이 여부와 관계 없이 나쁜 예후인자와 연관이 있었으며, *NRAS*와 *KRAS*의 mRNA 발현양은 *BRAF* 돌연변이 군에서 나쁜 예

후인자와 연관이 있었다. 향후 갑상선유두암의 예후 예측에 있어 *BRAF* 및 *RAS* mRNA 발현양 분석이 도움이 될 수 있을 가능성을 확인하였다.

주요어: 갑상선암, 갑상선유두암, BRAF, RAS, 예후인자

학번: 2015-30565